THE JOURNAL

OF

CANCER RESEARCH

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APPOSITIONAL GROWTH IN CROWN-GALL TUMORS AND IN CANCERS[1]

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Fifteen years ago some sections were cut for me from a tumor which I had produced in the cortex of a young tobacco plant by means of a single needle-prick introducing the Paris daisy strain of *Bacterium tumefaciens* Sm. and T. This was a young tumor, that is, one only three weeks old, and the sections were cut and stained in series. In the rush of other work these sections were overlooked and as they had been stained with a fugitive stain they faded so that when I came to examine them some years later there was a shoal of pale tissue surrounding the tumor which I could not in the least interpret. The cover-slips were removed by soaking in xylol and the sections restained, whereupon I was face to face with a new phenomenon (new to me). I had before me what cancer specialists have called conversion of normal cells into tumor-cells by *apposition*, that is by contact of the diseased with the normal, the "shoal" proving to be a 0.5 mm. wide layer of cells intermediate in character between the normal cortex-cells and the tumor-cells. By intermediate I mean midway in size and affinity for tumor-stains, and showing various transition stages including here and there a surrounded unchanged cortex-cell. (See this Journal, vol. i, 1916, no. 2, fig. 78).

I was much impressed by the phenomenon and in searching through other boxes of sections I found evidence of it in various tumors due to *Bacterium tumefaciens* including some produced in the cortex of the Paris daisy. The phenomenon is also shown

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on plate 29 of Bulletin 213 (l.c.), published in 1911, but the evidence in this figure had not then specially attracted my attention and is not mentioned in the text.   That there is here conversion of normal cells by apposition rather than invasion of normal tissue by cells growing out of the originally infected cells, or than simply an irritation-response of the host-cells which never passes over into tumor-tissue, there can be no question whatever for the cells have not changed places but the change has occurred *in situ* by the conversion of large cells wholly normal into congeries of small cells having all the characteristics of tumor-cells and visibly surrounded in many cases by the stretched wall of the original cell.   An irritation-response that does not pass over into tumor tissue proper also occurs in the vicinity of many crown galls, viz., an overgrowth of wood and bark, due to the stimulus of an extra amount of food moving in the direction of the tumor in greater quantity than it can use, so that some part of this excess either never actually reaches the tumor or oozes backward from it into the adjacent tissues, which are thereby incited to excessive growth; but this is something quite different from the response we are here dealing with because the cells of the hyperplasia in the one case have normal arrangement, normal staining properties, and function more or less normally whereas the cells in the other (the appositional growth) are smaller more or less disoriented and stain and behave like tumor-cells. For figures showing thickening of the wood entirely outside of the tumor but influenced by it see Bulletin 255 (l.c.), plates 25, 62A and 63, or An introduction to bacterial diseases of plants, fig. 319, subs 5 and 6.[2]   These proofs should convince any one that the phenomena here described are not the same although both kinds of growth are brought about by the presence of the tumor.

In 1916 I tried to reproduce the phenomenon of growth by apposition in tobacco-tumors, using the hop-strain of the crown-gall organism and single needle-pricks as before, but the gall was growing very slowly when collected and I got nothing com-

[2] See also my paper in Phytopathology, 1922, xii, pp. 265–269, pl. xviii.

parable to the earlier results which were produced, it will be remembered, with another strain of the organism.

In 1920 I repeated the experiment once more using, as in the first instance, the Paris-daisy strain for the inoculations. These inoculations were on the stem (cortex) of two young, growing tobacco plants by means of single needle-pricks in the manner shown on plate 1A. The aim in all cases was to confine the punctures to the cortex, making them as shallow as possible. In some instances it is likely that the outer phloem may have been reached but not the wood or pith and yet the wood is split open by the growth of the tumor-tissue and the pith is invaded. The tumors were collected and fixed in Carnoy's fluid (one-quarter glacial acetic acid, three-quarters absolute alcohol) at the end of three weeks. Sections in series have now been cut and stained from 15 of these tumors, and every one shows on some part of its periphery the same phenomena observed in the tumor of 1907. All the tumors when collected were increasing in size and in many cases this increase had been so rapid that remoter tissues were crushed. This expansion was partly from continued division of deep tumor-cells (as shown by an occasional mitosis) but also and chiefly from peripheral growth, *i.e.*, by conversion of neighboring normal cortex-cells (young cells, be it remembered) into tumor-cells, as shown in tangential section and in cross-section on plates 6, 7, 8, 9, 10. See especially plates 3B and 4, where the crushing is confined to the tissue bordering those lobes of the tumor which show marked appositional growth. This particular localization of the crushing, indicating excessive pressure, has been observed also in other tumors of this series (plates 2B, 10, 23).

Not in one only but in all of these galls there is the plainest evidence of tumor-growth by *apposition*, that is, peripheral extension of the tumor further and further into the cortex by the conversion of the adjacent normal cortex-cells into tumor-cells. One cortex-cell may give rise to a hundred or more tumor-cells. The sections show only a narrow periphery of cortex-cells undergoing conversion into tumor-cells (generally only a thickness of 0.5 mm. or less) but with the conversion of these

cells a new and remoter series of cells is subjected to the influence of the tumor with the same result, provided of course there has been no crushing, the tumor increasing in size as long as conditions for its growth are favorable.

Beyond the tissue in process of active conversion is a variable width of cortex-cells which are *larger than the normal cortex cells*, often twice their diameter but otherwise apparently nearly normal. For evidence of this plates 1B and 5 may be compared with plate 1A. This may also be seen clearly in 2A, 2B and 3A, and more highly magnified and very strikingly on plate 8. These cells have large nuclei and are dividing more freely than the normal tissue but not nearly as rapidly as the active transition tissue, and they are not different from normal cells in their orientation, in their intercellular aeration or in their relation to stains. These enlarged cells of the cortex are perhaps only cells stretched by proximity to the growing tumor, or enlarged and dividing on account of a more abundant water-supply, and yet we cannot say that they are not under a more direct influence of the bacteria which are the cause of the tumor. They are indicative at least of the nearby presence of tumor-tissue, quite as much as are the elongated glands surrounding a stomach carcinoma. The cortex in this region (round about the tumor) is often twice as thick as the normal cortex without having any more or at least many more cells in its structure. This may be seen very clearly from plates 2 and 3, prepared from 3 tumors where the enlarged cells form a sort of cushion on which the tumor rests.

I am the more inclined to publish my observations on appositional growth in these plant-tumors because of very positive statements by many cancer specialists, from Waldeyer, Cohnheim and Virchow down to Hauser, Krompecher, v. Hansemann, Petersen, Cornil, Fabre-Domergue, Menetrier, and others, as to the occurrence of appositional growth in carcinoma, and because in many respects crown galls are better adapted to the study of this phenomenon of growth by apposition than animal tumors, not only because we know them to be due to an intracellular schizomycete so that there is a definite reason for such growth, but because they can be reproduced at will and collected for

examination at any period of growth, and finally, because there are no migratory cells to confuse the picture.

Under dominance of a theory which has required them to ignore or explain away the plainest phenomena looking toward parasitism, Ribbert and his followers have denied the occurrence of growth by apposition in cancer. Cohnheim's theory of cancerous growth from misplaced embryonal tissue (cell-rests) having been abandoned, Ribbert's "nipping-off" theory was substituted, that is, growth of cancers wholly out of themselves, beginning in a fragment of epithelium dislodged by inflammatory connective tissue disturbances, or by trauma, and buried in the deeper tissues where it acts as an irritant and where it becomes converted into malignant tissue, but with the *How?* or *Why?* of its conversion remaining always unexplained. Ribbert at first maintained the origin of cancer from a single cell or cell-group and explained the appearance of islands of malignant tissue around the parent-tumor as due to deep strands of tumor-cells from the parent-tumor which, turning outward and upward, formed contacts with the epithelium immediately around the parent-tumor and thus gave the deceptive appearance of independent small growths. But Ribbert's theory also being now in less favor than formerly because frequently contrary to observed phenomena (Krompecher's, Petersen's, Adami's (1), and Cullen's, for example) there is once more the possibility of interpreting the undenied phenomena in consonance with what I shall here demonstrate to be true of crown gall, an indisputable bacterial tumor with various resemblances to malignant animal tumors.[3] Because of the importance of the subject I shall cite

[3] A plant-tumor of bacterial origin. Erwin F. Smith and C. O. Townsend. Science N. S., 1907, xxv, 671–673. Also a German paper with the same title in Centralb. f. Bakt. etc., II Abt., 1907, xx, 89–91.

The etiology of plant tumors. Erwin F. Smith. Science, n. s. 1909, xxx, 223.

Crown gall of plants. Erwin F. Smith. Phytopathology, 1911, i, 7–11, with 2 plates.

Crown gall of plants: Its cause and remedy. Erwin F. Smith, Nellie A. Brown and C. O. Townsend. U. S. Dept. of Agric., B. P. I. Bull. 213, Washington, Government Printing Office, 1911, with 85 figures on 36 plates.

The structure and development of crown gall: A plant cancer. Erwin F. Smith, Nellie A. Brown, and Lucia McCulloch. U. S. Dept. of Agriculture, B. P. I. Bull. 255. Washington, Government Printing Office, 1912, with 144 figures.

some of the views of leading oncologists *pro* and *con*, premising
that the whole subject from the human and animal side should
be worked over again using only primary tumors, *acting on
tissue of the same type*, and preferably young tumors arising in
columnar epithelium.

Ribbert's view, as set forth in chapter V of his last work
Das Karzinom des Menschen (2) is as follows:

From the microscopic behavior of cancer it cannot be doubtful that
the parts invaded are for the most part destroyed. Where the cancer
has fully developed, we cannot demonstrate the previously existing
tissue. But the naked eye might be deceived. For the thought is
near, from the microscopic appearance, that the bordering parts *may
have been converted into the carcinoma* and in this way have disappeared
where the tumor is found.

On some resemblances of crown-gall to human cancer. Erwin F. Smith.
Address as retiring President of the Bot. Soc. of America. Science, n. s., 1912,
xxxv, 161–172.

Le Cancer est-il une Maladie du Règne végétal? Erwin F. Smith. 1st
Congrès International de Pathologie Comparée. Paris, October, 17–23, 1912.
Tome II, pp. 984–1002. Also a separate, 19 pp.

Cancer in plants. Erwin F. Smith. Proceedings of the Seventeenth Inter-
national Congress of Medicine, held in London, August, 1913, III, 18 pp. Also
a separate.

Studies on the crown gall of plants: Its relation to human cancer. Erwin F.
Smith. Jour. Cancer Res., 1916, i, 231. No. 2, Pls. xxv, 82 figs.

Further evidence that crown gall of plants is cancer. Erwin F. Smith. Ad-
dress before the Washington Academy of Sciences. Science, n. s., 1916, xliii,
871–889.

Crown-gall studies showing changes in plant structures due to a changed
stimulus. Jour. Agr. Res., 1916, vi, 179–182, with 6 plates.

Mechanism of tumor growth in crown gall. Jour. Agr. Res., 1917, viii, 165–186,
plates 4–65.

Embryomas in plants produced by bacterial inoculations. Erwin F. Smith.
An address before the Johns Hopkins Medical Society. Bull. Johns Hopkins Hosp.,
1917, xxviii, 277–294, with 116 figures. Also a repaged separate.

Undersøgelser vedrørende nogle svulstlignende Dannelser hos Planter. [In-
vestigations concerning some tumor-resembling growths in plants.] C. O. Jensen
Kgl. Veterinaer-og Landbohøjskoles Aarsskrift. Serum Laboratory no. liv.
Copenhagen, 1918, 1 colored plate, and 17 figures in text.

An introduction to bacterial diseases of plants. Erwin F. Smith. Philadel-
phia and London, 1920, part III, chapter 14; and part IV, chapters 3 and 4, with
167 figures.

Effect of crown-gall inoculations on Bryophyllum. Jour. Agr. Res., 1921,
xxi, 593–597, 10 plates.

This was the assumption formerly for many tissues.   It was believed to have been demonstrated by histological investigations that other cells reached by the carcinoma were converted under its influence into cancer cells, that these consequently had an essential part in the spread of the tumor.   *This view, if it could have been accepted as true, would have been of great importance.*   Because from it would follow, that to the growing cancer *infectious characters* must be ascribed either dependent on the presence in the cancer of a parasite, or resting on specific chemical products produced by the tumor (p. 191).

It is remarkable that this view should have been maintained so long. One could understand it, if it related only to the acceptance of the view that cancer stimulates the bordering cells into growth. . . . .   But that one should think the cells of the carcinoma brought those of the other tissues not only to multiplication but also made out of them genuine carcinoma cells, this shoots wide over the mark (p. 191). . . .

Men assumed and still assume that epithelium of like origin can take part in the growth of the tumor, that it is drawn into a cancerous proliferation and so the carcinoma grows.   To this view I have been opposed for almost two decenniums.   *It is unquestionably false.*   But in spite of all my efforts and those of Borrmann who has ably supported me, it is not yet completely overthrown (p. 215). . . . .

Really, for the explanation of the growth of cancer in general this erroneous view could very well be discarded.   For even if here and there an appositional growth actually occurs, it can have only a negligible effect upon the spread of the tumor. . . . .

This is, therefore, not the ground which has led to the maintenance of the old doctrine.   Decisive rather have been views on the origin of carcinoma (p. 215).

Ribbert speaks of the fact that the beginnings of carcinoma cannot be found often enough for study, and in their place men have hoped on the borders of a carcinoma in the same type of epithelium to find and study equally well its beginning stages.

My doctrine[4] that cancer grows only out of itself was naturally disagreeable to views of this sort *und man bemühte sich mich zu widerlegen* (p. 215). . . . .

Perhaps one would not have taken the great trouble to seek out those cellular conversions, if he had had a clear conception that with

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[4] Doctrine not original with Ribbert but borrowed from the Frenchman, Bard.

the discovery of cancer-like cells newly arisen out of the neighboring epithelium, *the difficulties of explaining the genesis of carcinoma would be only so much the more increased.*[5]

How then should the cell metamorphosis take place?   The one meaning could be this, that from the cancer infectious influences proceed to the neighboring cells, through which these cells are biologically changed. But this view could be maintained only so long as one still thought the cancer a parasitic disease—*and even here without any foundation because parasites never change cells but only injure them.*   [The italics are mine, and the plates I show are a sufficient refutation of this statement.] But today, when we have universally given up the infectious theory of cancer there can be no more talk of any such explanation (p. 216).

Aber es ist ja gerade das wichtigste Ergebnis aller meiner bisherigen Untersuchungen und meiner Darstellung im Abschnitt V dieses Buches, dass die Anschauung, der Krebse wüchse durch stets erneute Apposition sich Krebsig unumwandelnder Epithelien, falsch ist.   *Der völlig entwickelte Krebs wächst immer nur aus sich heraus* (p. 482).

Ribbert maintains that no outside influence, parasites for example, or any symbiosis, can possibly induce cell-proliferation:

Es ist aber natürlich ein Fehler, das, was man bei der Symbiose sonst nicht beobachtet, auf das Gebiet des Krebses wilkürlich zu übertragen.

Von den meisten Seiten wird denn auch die Symbiose nicht zur Erklärung herangezogen.

Man begnügt sich damit, den Parasiten einen formativen Reiz auf die Zelle ausüben und sie so zur Wucherung bringen zu lassen.   Aber gibt es formative Reize?   Das ist eine alte Streitfrage, zu der auch ich mich wiederholt geäussert habe.   Ich bin in Übereinstimmung mit Weigert zu einer Verneinung der Frage gekommen und habe das besonders an zwei Stellen (Wesen der Krankheit und Deutsche Med. Woch., 1910, nr. 40) ausführlich begründet.   Hier muss ich auf die damaligen Ausführungen verweisen.   *Es giebt Keine formativen Reize in dem Sinne*, dass durch irgend welche Einwirkungen die Zellen direkt zum Wachstum gebracht werden könnten.   Wachstum kann immer nur auf Grund der dazu stets vorhandenen Fähigkeit ausgelöst werden, es kommt also immer auf indirektem Wege zustande, es fallen irgend

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[5] On the contrary from an etiological standpoint they are tremendously simplified and the phenomena are brought into correlation with what occurs in the plant.

welche Hindernisse, Hemmungen, Spannungen fort und dann wächst
die Zelle (385–386). .   .   .   .

Wie wir also die angenommenen Parasiten auch wirken lassen wollen,
ob durch Aufhebung der intrazellularen Spannung oder durch 'karzi-
nomatöse Umwandlung' ('Degeneration'!) oder durch Symbiose, in
keinem Falle leisten sie uns irgend etwas für das Verständnis der
Krebsgenese (pp. 386–387).

To all of this we may reply in Ribbert's own words:

Was nützen uns alle Spekulationen?   Ohne Tatsachen kommen wir
nicht weiter (p. 461).

The best defense of Ribbert's theory is by Borst in Die Lehre
von den Geschwülsten (3), and from this I cite at some length
making my own translation as in the other cases.

Then the further growth of the tumor takes place not by continual
conversion (Einbeziehung) of the normal surrounding tissue into the
same sort of degenerative growth, but the tumor develops out of it-
self. .   .   .   .   If I speak here positively it is because I have given
special attention to this point in my own extensive studies. *Even now,
most specialists hold to the doctrine of a peripheral growth in carcinoma*
[the italics are mine] in the sense that continually, close around the
tumor, cells of ordinary and of glandular epithelium, hitherto normal,
undergo a cancerous change (Hauser, Beneke, and others).   Formerly
this view of the growth of carcinoma (and of tumors in general) was
the universal one.   It was conceived that an agent of unknown nature
inciting to proliferation was distributed in the periphery of the carcin-
oma and that this agent changed the hitherto normal cells into a
cancerous growth; sometimes this agent was pictured as a fluid men-
struum, sometimes as a mass composed of the finest granules (Gussen-
bauer), sometimes the cancer cells themselves were supposed to contain
a substance which could exercise this growth-irritation on the surround-
ing cells.   Subsequently, making use of the data of bacteriological and
parasitological investigations, parasites were thought of as possible
causes.   Beneke speaks in a general way of an 'Umstimmung' of the
normal cells, of a reciprocal influence of the cells which makes possible a
transfer of the 'Blastomatosis' from cancer cells to physiological cells.

The opinion that in cancer progressive growth takes place in this
way, that the bordering parts are continuously drawn into the degen-

erate growth ('Nachbarinfektion') was based chiefly on histological transition appearances (Borst, Bd. II, 697–698).

Borst does not deny the existence of such appearances in the vicinity of cancers but he denies that they are transition stages. He thinks he has another "simple and plausible explanation." He says the same changes occur in inflammatory conditions and in regenerative new formations, especially in regenerative processes complicated and disturbed by inflammatory processes.

Under these conditions very striking atypical epithelial growths arise, which Friedländer, more especially, has fully described, and which have absolutely nothing to do with cancerous degeneration. . . . . In the course of chronic inflammations the regeneration of common epithelium and glandular epithelium often gives pictures strikingly like cancer (Borst, II, 698–699).

But along with disputed doubtful spots on the edge of a carcinoma we find other spots in which plainly beyond any doubt entirely unchanged, normal epithelium lies close up against carcinoma parenchyma without a trace of progressive change to be observed in the former, on the contrary often enough disintegrating (*rückläufige*) metamorphoses are to be found. The proliferative processes in the common and glandular epithelium just beyond the periphery of carcinomas are therefore of the same order as the multiplication of the connective tissue, of the blood vessels, of the bony tissue, etc., which we have learned to regard as reactions of the connective substance against the penetrating carcinoma. Hence the fact, that in the tissues roundabout a carcinoma through the secondary occurrence and fusing of parenchyma masses of the carcinoma with neighboring, preexisting epithelial masses which are normal or in process of a growth reaction, pictures arise which might be interpreted as a transition of normal epithelium into cancerous, and many times have been so interpreted, as we have already mentioned. The following point is also important: many carcinomas more or less completely resemble the mother-tissue, and often to such a degree that we can find all sorts of transitions from very crude (stumperhaften) imitations to the formation of almost typical forms in one and the same tumor; if we add that these forms recalling the mother-tissue (*e.g.*, glandular tubes) also recall or may recall by their grouped arrangement and fusion, the coarser structure of the mother-tissue (*e.g.*, glandular lobes that occur in cancer of the breast) it is easy to

see how the deception would arise that one has before him nothing less than normal structures (*e.g.*, gland-lobules) in process of cancerous conversion. The absence of excretory ducts in such groups of cancer bodies, the lack of a membrana propria, the betraying irregularity in the formation of the cancer-body in spite of all other resemblances, its extraordinary multiplication, and so forth, will serve to prevent confusion. In consideration of all these circumstances and especially with the use of the finer histological technic which shows us clearly the difference in nuclear and protoplasmic structure and in the type of mitosis, we shall not in most cases be deceived into believing that on the periphery of a carcinoma we have a transition from normal epithelium into a carcinomatous parenchyma (Borst, II, 699–700).

Borst says it has been established that endothelial cells are not converted into cancer-cells and that when a cancer in one sort of epithelium impinges on another sort of epithelium there is no conversion of the latter. But this his opponents except Carl Gussenbauer (4) also admit.

With the above limitations the importance of the growth of carcinoma by a peripheral change of normal parts is greatly reduced;[6] it is therefore certain that *by far the greater part* of every carcinoma grows out of itself, and that only where the carcinoma abuts on cells of like origin may a cancerous conversion of normal tissue take place. But also against this last cardinal point of the theory of 'tissue infection' in cancer all the above mentioned considerations are opposed (Borst, II, 701–702).

Borst's book is very attractive and he seems to be a fair debater since he cites his opponents and does not distort their views. I think, however, he is swayed a good deal by his preconceived ideas as to the cause of cancer. If he thought it due to a parasite, then he would interpret the admitted facts in quite another way. Most of his above mentioned sources of error are such as would apply to the interpretations of tyros rather than to those of experts of a like experience and reputation with himself.

[6] Here we may distinguish clearly, as Borst does not, between clinical importance and etiological importance.

For older views concerning growth of tumors by apposition I should like to cite Virchow and for more recent views Hansemann, Hauser, Krompecher, Petersen, Menetrier and others.

Virchow (5) writes as follows:

Earlier than the stage of the formation of the formative cells or *primordial cells* [of the tumor], as they have also been called, a whole series of changes have taken place, and the tumor does not begin where the formative cells lie, but there where the first change in the mother tissue took place [or, to use a modern phrase, in the precancerous stage]. . . . .

From this we see that a true *boundary between the tumor and the mother tissue* is not present; at the beginning the tumor is in complete and intimate connection with the mother tissue (Vol. i, p. 93).[7]

Hansemann in 1897 (7) writes as follows:

It is in general plain even from a macroscopic observation that the primary tumors end diffusely in the surrounding tissue, while the secondary tumors are sharply delimited from the organ-parenchyma (p. 124).

Hansemann speaks of a collateral hyperplasia in the vicinity of primary tumors. This is a common occurrence, as every one knows who has studied cancer. He says:

If we begin first with the primary tumors, we shall see that in the vicinity of such a tumor, the tissue out of which the tumor has developed, and also the related tissue, becomes hypertrophied with great regularity, although there are some exceptions. In the vicinity of a cancer of the skin (cancroid), of the mucous membrane and of the oesophagus the epithelial margins are widened and elongated, and the papillary bodies are enlarged. The sweat glands and sebaceous glands may also be involved and this general hypertrophy gradually extends outward. All the layers of the skin are involved in this hypertrophy, the germinal layer, the rete, and also the cutaneous layer in case of epidermoidal formations. Also the mucous membranes with ciliated epithelium and cylinder epithelium become thickened or more often

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[7] For many interesting figures of edges and early stages of cancers showing this see Thos. S. Cullen: Cancer of the uterus.[6]

become epidermoidal in the vicinity of a primary carcinoma, the latter especially if they have become cancroid through cell-variation [by cancroid he means a keratinizing cancer]. In stomach and intestinal cancers we see the gland-tubules in their vicinity elongated and also in cancer of the uterus and eroding carcinoma of the portio there is a growth of the glands in the vicinity into long tubules. These collateral growths are often of such dimensions that they constitute special tumors. On the outer skin, and especially in the larynx, verrucose thickenings and great warts arise, which might be taken for benign growths if they alone were excised for the examination. In the intestinal tract, in the uterus and in the bladder, papillary cauliflower-like excrescences arise which may also lead to confusion (pp. 125–126).

But where the collateral hyperplasia arises, it passes over so gradually into cancerously changed tissue, that often under the microscope we cannot tell exactly the cells which mark the boundary between the two conditions. Ribbert (pp. 143–150) has called attention to the interesting fact that carcinoma of the skin and mucous membranes, of the breast, etc., proceeding from a center may grow outward and fuse with the epithelium of the mother-organ, so that it may appear in close union with the latter. For this reason the carcinoma [at the point of fusion] may seem to have begun independently, whereas really it is only a secondary growth. I can fully confirm this observation of Ribbert, but I am not of his opinion that we may generalize this fact so as to apply it to all cases. Indeed, I believe that Ribbert's account applies only to a minority of the carcinomas, while in the greater number of cases an actual conversion of hyperplastic tissue into tumor-parenchyma occurs. Upon this collateral hyperplasia depends the observation that primary tumors both to macroscopic and often also to microscopic observation end rather diffusely in the surrounding tissues. In this they are quite distinct from the metastases, because the latter usually have sharp boundaries (pp. 126–127).

Rokitansky taught that the center is the youngest part of a tumor but Virchow (8) showed that the periphery is the youngest part (it is also the youngest part of a crown gall) and taught that one must study the margins of tumors if one would learn how they develop. Concerning this Hansemann remarks:

Up to this time all writers on cancer have followed this dictum and they were the more inclined to do so because everyone believed that a

primary tumor grew in this manner, that out of its vicinity an ever increasing number of parts were converted into the tumor mass. On the contrary quite recently Ribbert (Hugo Ribbert: Pathologische Wachstum der Gewebe, Bonn, 1896) has offered objections. . . . . It must be recognized that there are such appearances as Ribbert describes and that he is correct in maintaining, for his cases, that the carcinoma proceeds from the surface and then from the depths again grows back to the surface, in order to appear here in union with the normal elements of the parenchyma. . . . . Ribbert's mistake lies in having generalized his conclusions and in declaring that there is no other method of propagation. Indeed, I maintain that this other way is the principal way. I possess a whole series of preparations of epidermal carcinomas, of stomach and intestinal cancers, etc., in which I can demonstrate it indisputably, and in which any fusion in Ribbert's sense is out of the question (pp. 156–157, 1st ed., and 199–200, 2d ed. 1902).

Hansemann sums up as follows:

We must, therefore, I believe, maintain that malignant tumors arise from a restricted spot, even from a single cell, and these enlarge out of themselves, but that also they may arise contemporaneously or succedaneously over large areas and besides their growth out of themselves, which naturally always occurs, may grow by confluence and by apposition (p. 157).

Nobody yet has seen to a certainty the very earliest stages of carcinoma, even Ribbert, who claims his tumors alone as sufficiently small and all others as too far advanced, to decide the question. Nevertheless, it is only possible to recognize a thing as carcinoma if it has plainly the structure of the latter; then, however, it is a definite structure and not a becoming. So long as it is still in process of originating, we cannot know what it will become when it has grown farther (p. 158).

No man has ever seen a tumor arise under the microscope (p. 154).

In 1910 von Hansemann (9) also wrote as follows:

Von grosser Bedeutung sind die Beziehungen der bösartigen Geschwülste zur Nachbarschaft. Wenn eine Geschwulst wächst, so kann sie auf die Nachbarschaft in verschiedener Weise einwirken, entweder verdrängend oder auflösend, oder wucherungserregend. . . . (p. 14)

Besonders bemerkenswert ist der Umstand dass Karzinome, die von

irgend einer Epithelschicht ausgehen, in der Nachbarschaft das gleiche Epithel zu Wucherungen anregen, und dadurch unterscheiden sich primäre Geschwülste eines solchen Epithels von sekundären (p. 16).

In opposition to Ribbert, Hauser (10) also found in cancer of the stomach and large intestine, in about 80 per cent of his cases, no metastases of the epithelium when there was peripheral conversion of the glands into cancer (p. 492). He says:

On the other hand as I have shown [pages and plates cited], in these same stomach and intestinal cancers primary changes of the gland epithelium occur which are unquestionably specific for carcinoma, since they are observed nowhere else, so that out of these changes alone cancer can be diagnosed (p. 492).

The same thing occurs, he says, in cylinder epithelial cancer of the uterus (492).[8]

Hauser's paper is accompanied by one plate containing two figures, both of which are tremendously interesting, especially his figure 2 which shows, enlarged, the transitional border line of an intestinal cancer, i.e., conversion from cylinder-cell normal gland tissue to a kind of irregular squamous-cell cancerous tissue. Here the epithelial cylinder cells in the outer part of a gland-tubule are normal, the middle cells of this tubule show transition forms, while the inner half of the tubule is wholly cancerous, has fused with the cancerous cells of an adjacent tubule and has broken through the muscularis mucosae, as is clear from his figure 1, which gives the orientation of his figure 2 (fig. 1 of my copy).

Excluding many cases of carcinoma solidum and gelatinosum, we find these specific cancerous changes of the glands of the mucous membrane especially in that form of carcinoma cylindro-epithel adenomatosum, in which in consequence of active multiplication the one-layered epithelium becomes several-layered while at the same time the epithelial cells themselves experience profound morphological changes. They lose completely their cylindrical form, become exquisitely polymorphous, resembling the cells of the many-layered pavement epi-

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[8] In this connection see Cullen (l.c.) figs. 138, 196, 198, 209, 214, 230, 233, 234, 235.

thelium, the nucleus becomes larger and often extremely chromatin rich, the protoplasm appears fine granular, the normal mucin (Schleim) has everywhere ceased, so that even in the large intestine there is not anywhere any more production of beaker cells, and the whole epithelial layer shows an intensive staining. . . . . .

All these profound changes of the glandular epithelium, or of the glands of the mucous membrane, which we have to consider as a specific cancerous conversion (Entartung) of the glands of the mucosa, because it occurs exclusively in carcinoma, we may observe in suitable objects with fully preserved membrana propria, and at a time when the cancerously degenerated gland-tubules have not yet anywhere broken through the muscularis mucosae . . . . (p. 493).

FIG. 1. AFTER HAUSER. SEE TEXT

Finally, as to the question whether it is possible that a carcinoma may arise through the metastasis of normal epithelium alone, that is, without change in its biological characters, such a possibility according to my notion is wholly excluded. . . . . It is in opposition to the normal laws of growth.

The cancerous development can rest therefore only on a fundamental change in the biological peculiarities of the epithelial cells. Only such an hypothesis can explain the fact that normal body-cells in their later generations acquire definite parasitic peculiarities (pp. 496–497).

For the specific cancerous conversion of epithelium, at least in cylinder-epithelium carcinoma, we have a definite morphological standpoint: The loss of physiological function, the change of typical cylinder

epithelium into several layered polymorphic epithelium, the changed size-relations, especially the very frequently observed enlargement of the cells with enlargement of the nucleus at the same time and increase in the chromatin contents, further the changed form of the mitoses, the very abundant appearance of hypochromatic, hyperchromatic, asymmetrical and multipolar cell-division figures and finally the enormous capacity of the cancer cells for multiplication which is clearly connected with a certain feebleness and shorter life—all these changes, very pronounced in many cases, in my opinion point clearly to the fact that the cancer cell has become another cell, morphologically and biologically, from the mother cell from which it has descended, that an *Entdifferencirung* or *Anaplasia*, as Hansemann calls it, in short a 'specific cancerous degeneration' has taken place (pp. 497–498).

As to the impulse causing this cancerous degeneration of the epithelium, we know nothing (p. 498).

Hauser also refers repeatedly to growth by apposition in his book on cancer of the stomach and intestine (11). Here he says:

For the further growth of the primary cancer proceeds generally in this manner, that while on the one hand the epithelial growth which has broken through into the submucosa pushes outwards in all directions and penetrates ever deeper into the tissue, on the other hand the cancerous degeneration of the glands of the mucous membrane on the periphery of the cancerous mass progresses continually, so that, over and over, new glands, cancerously degenerated, break through into the submucosa. . . . . This centrifugal growth of the cancerous new formation brought about by apposition, is in many cases of carcinoma of the stomach and intestines, especially in the simple and scirrhous forms, the preponderating one. . . . . .

The cancerous degeneration of the glands of the mucous membrane, continually progressing on the periphery of the primary tumor, appears in most cases to persist uninterruptedly to the end of the cancerous disease, that is to the death of the individual. For otherwise it is inexplicable that on the periphery of the carcinoma, whether the latter be large or small, ulcerated or not, almost without exception we find glands which show the most varied stages of cancerous degeneration even to penetration of the deeper layers of tissue. In many cases, this progressive disease of the glands appears to be quite uniform in all

parts of the periphery so that under microscopic investigation of numerous and suitable spots of the carcinoma's edge everywhere the same behavior is to be observed. But also we frequently find a more or less great irregularity in the progress of the cancerous gland degeneration in that while the same proceeds vigorously in some parts of the cancer it appears to have ceased in other parts, either wholly or partially [see plate 4A of this paper], at least for the time being (pp. 91, 92).

Krompecher writing on basal-cell cancer (12) comments as follows on the subject in question:

The boundary between the carcinoma epithelium and the surface epithelium is not distinct in the greater number of the basal-cell cancers; *die normalen Epithelzellen gehen vielmehr ganz allmählich in die Carcinomzellen über.* Such a gradual transition where a union with the basal epithelium was demonstrably altogether objection free, I found 14 times out of 16 cases (p. 71).

Krompecher mentions especially 8 cases where there are islands of cancerous tissue around the primary tumor and says: that here any union of the carcinoma epithelium and the surface epithelium in Ribbert's sense could be entirely excluded (p. 71).

Likewise I found in keratinizing cancers and also in the greater number of basal-cell carcinomas a gradual transition of the cell-sorts so that actually it was not possible to say where the carcinoma commenced (p. 72).

He cites other authorities against Ribbert and his students (Hansemann, Hauser, Lubarsch, Nothaft, Petersen) and says:

How plainly the strife turns about the question whether in beginning carcinoma the connective tissue or the epithelium plays a superordinate, a coördinate or a subordinate rôle (p. 77).

I must also cite Petersen since I conceive that some of his findings, like Krompecher's, also have a direct bearing on what I shall say when I come to discuss the discrete small tumors which I have found in the pith of some of my preparations.

Petersen discusses Ribbert's views in several long papers dealing with cancer. I quote as follows from his Beiträge zur Lehre von Carcinom (13).

On the other hand how little even the purely morphological questions of carcinoma are settled, will best be shown by the fact that seven years ago an investigator of Ribbert's importance turned everything pretty much topsy-turvy which had hitherto been accepted concerning the histogenesis and growth of carcinoma. Since then there has been a lively strife back and forth over these questions (p. 545).

According therefore to the theory of Thiersch and Waldeyer, each carcinoma begins with a disturbance of the boundary (Grenzverschiebung) between epithelium and connective tissue. Which of the two tissues causes this? Owing to the labors of Thiersch, Hauser, Hansemann and others, there is very little doubt that the epithelium is the active part: The carcinomatous growth would then depend on a fundamental change in the biological character of the epithelium, the primary cause of which remains in doubt.

Then comes Ribbert and says: Quite the opposite! The primary thing in carcinoma is always a connective tissue multiplication. This leads to the splitting off of epithelium and the epithelium thus separated from its organic union and the regulatory influence of the organism acquires the power of unlimited growth (p. 545).

Against this question of the *peripheral growth of carcinoma* Ribbert's opposition sets in most intensely. Here I must stop a moment to point out the fundamental importance of this question. Previous to Ribbert the growth of a carcinoma had been represented for the most part as developing, when once started, out of itself by *intussusception* but also growing by *apposition*, that is through the continuous cancerous conversion of the neighboring epithelium. The first way applied to the deeper growth, the latter to the peripheral spread of the tumor (p. 546).

With this last view Petersen agrees and shows figures to illustrate appositional growth. Of one of these figures he remarks:

In B and C of figure 2, then, the peripheral spread may be conceived as due to a cancerous conversion of the epithelium. And certainly this process must ordinarily progress continuously, so that the surface of the carcinoma always represents a fusion surface . . . . (p. 546–547).

If this proposition is true [Ribbert's view that the edges of large carcinomas are entirely unsuited for the study of the histogenesis of carcinoma and that only very small beginning carcinomas are suitable] then, as Hansemann says, the body of our histogenetic knowledge is destroyed; for what is a beginning carcinoma? Here the whole field

is thrown wide open to a subjective conception and the danger is near
that a tumor which will not fit the theory is either too large, that is
not any longer suitable, or too small, that is *not yet suitable*. This
question of peripheral growth occupies, therefore, since Ribbert's
first publications, always the center of the discussion (p. 549).

Ribbert's theory, at least as a whole, is not accepted by any im-
portant group. A series of microscopic observations have been brought
forward as incompatible with it; against its theoretical foundations
serious objections are also raised. But just as little can Ribbert's
theory in its totality be regarded as set aside (p. 557).

Petersen found in his third wax model[9] several (8 or more)
small completely isolated epithelial (cancerous) islands close
to the main body of the small tumor (see plates 22 to 27 of this
paper) and adds:

We get in many places the impression as if originally isolated islands
had united with the principal tumor during its further growth (p. 570).

One somewhat larger island was 2 mm. removed from the main
tumor. The cells of these islands which, he says, are to be con-
sidered as the first metastases, are much farther degenerated
than the body of the tumor. This is striking. They are horny,
often more flattened with very indistinct nuclei, as if degenerat-
ing (p. 570). His fourth model is a splendid demonstration
of the independent development of small epithelial cancers
around the primary center. Of these he says:

The entire remaining accessory centers ("Nebenherde") *c, d, e* and *f*
are completely isolated, especially *e* and *f* (p. 574).

He calls this a "reticular multicentric carcinoma" and says
the changes in the connective tissue are so slight that they cannot
be considered as a primary cause of the tumor. There was no-
where granulation tissue and nowhere lifting up of the epithe-

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[9] For those who do not understand this process it may be said that for a wax
model a tumor is cut in series and an enlarged wax model is then made of each
section indicating clearly the cancerous portion. These thin sections are then
superposed one on the other and thus a model of the tumor with all of its rami-
fications is obtained.

lium (p. 576). The tumor had clearly enlarged from the periphery outward by developing *discontinuously* one after another new cancer nodules around the primary tumor (p. 575) with which later, he thinks, the main tumor would have united.

Petersen says he has microscopic preparations of 330 skin cancers of which 130 were cut wholly or mostly in series. He also knows the history of all of the patients (p. 580). He thinks many skin cancers (the multicentric ones) arise in hair-bulbs, sweat glands or sebaceous glands, and these independent tumors may remain independent or afterwards fuse as the tumors grow (p. 581). Virchow also held this view and expressed it as follows (14):

As a rule, along with the mother tumor which may be growing slowly or not at all, at very different distances from it arise new foci which sooner or later unite with the mother nodule (p. 21).

Petersen continues:

We must therefore maintain that the vicinity of a multicentric carcinoma (wholly irrespective of the cancer cells here scattered) is more disposed to a new carcinoma than remoter places (p. 596).

After marshalling much evidence Petersen says:

A carcinoma in the sense of Thiersch and Hauser may arise through a *primary epithelial change*; the changed epithelium can grow uninterruptedly in the depths; there is no need, therefore, for any "nipping-off" in Ribbert's sense (p. 628).

Thiersch, who taught that carcinoma begins in the epithelium, held it probable that there is in the beginning of carcinoma a biological weakening of the connective tissue (p. 631).

Petersen, who does not believe in the parasitic theory, closes his long paper as follows:

Es kann daher sowohl wissenschaftlich für die Lösung dieses dunkelsten aller pathologischen Probleme, als auch praktisch für die Bekämpfung dieser furchbarsten aller Krankheiten nur von Vorteil sein wenn von verschiedenen Seiten her und mit verschiedener Fragestellung immer wieder von neuem, trotz aller Misserfolge, unermüdlich das Studium des Carcinoms in Angriff genommen wird (p. 651).

In this connection also Virchow wrote as follows at the close of his Cellular Pathology (15):

A pathological tumor in man forms in exactly the same way as does a swelling on a tree, whether on the bark, or on the surface of the trunk or on a leaf, where any pathological irritation has occurred. . . . . All of them depend upon a proliferation of cells just as abundant and often just as rapid as that which we see in a tumor of a proliferating part of the human body. The pathological irritation acts in both cases in exactly the same manner. . . . . The great importance which a knowledge of botany possesses for the pathologist also lies in this, that it enables him to discover in all these processes the existence of an inward correspondence in the whole series of vital phenomena, and to show how the lowest formations may serve to explain the history of the most perfect and complex parts.

All of which shows Virchow to have been a bigger and broader man, and a better pathologist, than some who have come after him.

Dr. Carl Ritter of the Surgical Clinic in Greifswald in 1901 (16) maintained the parasitic nature of cancer as follows:

Moreover, the whole theory that the tumor-cells are the parasite, is no explanation of the well-known facts; for the cause, whereby a [body-] cell is suddenly converted into a foreign parasite, is not in the least explained thereby (p. 175).

Ritter's strongest argument, perhaps, is that necrosis in cancer is not due to lack of blood supply but must be due to the gradually cumulative action of the products of cell-parasites. In infectious diseases the necrosis is always central and exactly so it is in carcinoma and sarcoma (p. 181). Jenny, he says, has pointed out that no one has offered a satisfactory explanation for the fact that the keratinizing process in tumors of this type is always central. Lange describes three zones in colloid cancer of the stomach, intestine, and vagina of which the outer is free or freest from degeneration, (p. 182). Ritter says all observers are agreed that the necrotic parts are central while the fresh parts with well-stained nuclei and mitotic figures are peripheral (p. 182).

Das Räthsel lässt sich meines Erachtens nicht lösen, ohne die Annahme eines fremden Virus, was an der Stelle der Verhornung diese Degeneration verursacht oder verursacht hat.

In gleicher Weise findet sich die Verkäsung und die gallertige Degeneration central, nicht am Rande . . . . (p. 182).

Es geht wohl aus dem Gesagten hervor, dass die Degenerationen sich ganz ausserordentlich leicht unter der Annahme einer Infectiosität der Geschwülste erklären lassen. Diese Erklärung ist die einzige, die im Stande ist, alle Erscheinungen bei den Degenerationen zu erklären.

Ganz anders ist dies aber bei der Metastasenlehre, die mit der Annahme einer Infection, wie es scheint, unvereinbar ist (p. 183).

Ritter's inability to explain metastases on any parasitic basis, something not so difficult now that we know the behavior of crown gall, leads him to call them in question. His chief argument is based on "the impossibility of distinguishing by cell-form" carcinomatous tumors in other organs from tumors derived wholly from connective tissue cells. Round, or spindle, or giant cell sarcoma, or endothelioma or perithelioma, and tissues capable of producing such tumor cells, occur in every organ (p. 183).

Often an endothelioma or a perithelioma may so closely resemble a carcinoma as to be mistaken for one. A whole series of tumors formerly diagnosed as carcinoma must now be referred to endothelioma. Krompecher has shown that the most malignant tumor of the testicle is an endothelioma yet he found no such tumor recorded in literature (p. 184).

To explain gall-secretion in a lung-tumor, derived from a primary tumor in the liver, Ritter is obliged to call it a malignant adenoma and separate it from carcinoma (p. 185).

His malignant adenoma differs from carcinoma in not having the epithelium in several layers. It has no polymorphism of cells, no solid masses (Zapfen). It retains glandular structure and function, but has unlimited growth, and the metastases have the character of the mother tumor (p. 189).

Ritter makes two pertinent conclusions, patent to anyone who has reflected much on this subject: First, it is not possible

to write the pathology of a disease correctly until we know its
cause; second, very likely the organism of cancer has already
been isolated and neglected.

In 1899 Prof. Dr. Vincenz Czerny of the Heidelberg Surgical
Clinic (17) said that sarcoma is so much like certain infectious
diseases that von Esmarch suggested that all of it might be of
syphilitic origin, and that actinomyces was long called osteosar-
coma till Bollinger discovered the cause. He thinks it probable
that many cases of malignant lymphoma and lymphosarcoma
are due to modified tubercle bacilli (p. 251).

Several dozen times in his clinic when cancer of the lower
lip has been excised along with the swollen regional lymph glands,
no malignant cells have been found in the swollen lymphatics,
only simple hyperplasia, yet in a clinical sense they were car-
cinomatous because, if left, there would have been a return of
the carcinoma (p. 257).

Im klinischen Sinne waren diese Drüsen also schon carcinomatös
inficiert, ohne dass man es schon anatomisch nachweisen konnte.

Menetrier in his excellent book on Cancer (18) takes the same
view as Hauser, Hansemann *et al*. He says under epithelial
cancer:

The increase by multiplication of its elements is indubitable. This
is established by the fact that the mitotic figures are confined almost
exclusively to the epithelial elements, the parenchyma, and not to the
stroma; but in the extension to neighboring parts it is necessary to
distinguish two possible ways: extension by *transformation* of like ele-
ments, and extension by *substitution* of cancerous cells in place of the
adjacent tissues.

Extension by transformation. The extension by transformation is the
most interesting to consider, because, even after the beginning phase of
the cancer, when the latter has already considerable dimensions, some-
thing that habitually occurs in the cases ordinarily under observation, it
enables us still to find and study the pathogenic process which has given
rise to a malignant neoplasm.

It is through a study of the borders of the cancer that we may find
the young lesions, still in formation. All authors to be exact, do not
admit this concept, and among the more recent, Ribbert and Borst

energetically oppose any such interpretation; for them cancer once formed, extends of itself, without transformation of neighboring elements. "The carcinoma, on the border," says Borst, "advances with its own troops and does not add to itself new soldiers at the expense of healthy tissue."

We think on the contrary that, in a certain number of cases, the cancer not yet escaped from the tissue or the organ in which it has begun may extend by transformation of similar elements, that is of elements of the same nature as those which have given birth to it, and which are subject to the same modifying and preparatory causes of the cancerous evolution . . . . (pp. 181–182).

Concerning cancer of the stomach he says:

As we approach the cancerous ulceration we see a rapid increase in the size of the glands. They form a layer in which the thickness of the glandular tissue is such that it is really a glandular tumor, an adenoma, this, however, without the hypertrophied glands having lost the fundamental characters of their structure. Their tubular conduits are elongated so that they may be 5 or 6 times as long as normal, but their walls are not broken, their proper membrane persists, and in their interior there is a continuous covering of cylindrical mucous cells, corresponding to the type of covering normal to the glands of the pyloric region, which is the region in question.

But this epithelium is also, itself, hypertrophied, as indicated both by the length of its cells and by its vegetative tendency. This while scarcely noticeable at first shows more and more distinctly as we approach the cancerous zone (fig. 8, from A to B). In all this adenomatous zone, the thick glandular layer remains sharply limited by the muscularis mucosae.

The hypertrophied glandular layer passes over directly into a layer which is clearly cancerous, formed of an infinity of tubes, irregularly shaped, lying without order in all directions, no longer recalling any glandular structure, and lined with a cylindric epithelium having its protoplasm quite uniformly colored throughout its length; it is the typical gastric cylindrical epithelioma, which occupies not only all the thickness of the mucosa, but extends into the depths of the subjacent layers after the more or less complete destruction of the muscularis mucosae (fig. 8C).

Between these two zones, the epitheliomatous zone and the adenomatous zone, a transition zone occurs (fig. 8D), passage from the

adenoma into the cancer, which, on the section that we have had drawn, appears to us to show phenomena strikingly demonstrative of the opinion we maintain of the continuity of the process of the adenomatous origin of cancer. At this point we see, in fact, a gland enormously enlarged in all its dimensions, hypertrophied and vegetating as to its epithelium and which nevertheless is recognizable as a gland. This appearance, moreover, grades through lesser deformations into perfectly typical glands of the region.

In its upper excretory part, the epithelial covering [of the gland] is identical with that of neighboring mucous glands, only more vegetative, as the sinuosities of its surface prove. In its deeper part, this covering, always continuous, but still more vegetative, as shown by the more sinuous line, takes on morphological appearances identical with those of the epithelium which constitutes the epitheliomatous tubes of the zone which is clearly cancerous. The gland, however, is complete, its covering is continuous, there is not any interruption of the epithelial layer, nor penetration of the epithelial masses proliferated from a neighboring region into the glandular cavities. There is no appearance of invasion of the gland by the epithelioma, but the appearance is clearly that of a transformation in place of the epithelium of the gland. Nearby, the cul-de-sacs of two glands, obliquely cut, show a similar transformation of the glandular covering, still more vegetative. And then in the cancerous zone, an epithelium provided with the same morphological characters is disposed in irregular tubes, representing a still typical but disordered proliferation, one in which the primitive glandular texture, the hyperplasial glandular walls of the adenomatous zone, have completely disappeared.

To sum up, these lesions appear to us characteristic of a cancerous formation due to transformation of the epithelial covering of the adenomatous glands. It does not correspond to an invasion by substitution of the proliferated cancer in place of the elements of the hypertrophied glands, as some have maintained, because in no place does one see a destruction of the glandular epithelium, which would have to take place in such a case, while we actually see the transformation, in place, of adenomatous epithelium into cancerous epithelium. And in the ulterior progress of the lesion, it is the non-epithelial gland-wall, the membrane proper, the connective framework, which is destroyed and disappears, while the emancipated epithelium continues to proliferate . . . . (pp. 183–186).

This mode of extension is found also in other varieties of cancers and we obtain pictures equally demonstrative in certain skin cancers, and notably in cancers of the lips, or even in cancers of the buccal cavity, when the examination is carried out on tumors not too old or too voluminous · . . . . (p. 186).

This mode of extension belongs essentially to cancers of hyperplasial, and especially of adenomatous or papillomatous origin; it is generally absent, on the contrary, in cancers of heterotopic [metastasial] origin, that develop habitually from cellular islands of very small size, which are rapidly transformed and become unrecognizable as soon as the cancer has attained notable proportions (p. 188).

Versé's statements (1908) as to the result of his researches on a wealth of material and covering half a dozen years are equally explicit. After going critically over Ribbert and Borrmann's views he comes to a contrary opinion, siding with Hauser. After examining 105 epithelial tumors in thousands of sections, he says (26):

An den Rändern älterer Karzinome kann nun eine weitere Umwandlung des Epithels vorkommen. Aber auch hier tritt die Änderung successive ein; es bildet sich erst ein cylindrisches indifferentes Epithel, aus dem durch immer weitere Proliferation die eigentlichen Karzinomzellen entstehen (p. 145).

Es ist sehr warscheinlich, dass die meisten Karzinome des Magendarmkanals aus Adenomen oder Polypen hervorgehen; jedenfalls ist ein adenomatöses Vorstadium anzunehmen (p. 158).

Die Karzinome entwickeln sich aus den Körperzellen ihres Trägers in folge einer allmählichen, am rande mitunter noch fortschreitenden Umwandlung des Epithels in primär erkrankten Organ. Durch den histologisch ganz exakt zu erbringenden Nachweis dieser langsam sich vollziehenden Epithelveränderung wird den Theorien, welche die Entstehung des Karzinoms aus einer direkten Einwirkung eines Mikrobions auf die Epithelzelle ableiten oder überhaupt die Krebse aus Implantationen Körperfremder Zellen hervorgehen lassen wollen, der Boden entzogen (p. 160).

Here he is speculating and his feet are off the ground.

Lubarsch's comments are also very interesting. Of the trend of opinion among cancer specialists in 1908, which he thinks was pushed too far, he says (24):

Here we must first outline the question what sort of material we may use to investigate the histological development of cancer. As is well known, the views have changed greatly in the last 10 years, owing to the unwearied activity of Ribbert. Previously it was believed that on the edges, even of large well-developed carcinomas, the origin of the cancer could be recognized, but this view is now as good as completely abandoned. The ruling dogma is that a cancer can grow only out of itself, that it never increases by apposition, and that only the investigation of so-called beginning carcinomas can give any idea of cancer development (p. 34).

Lubarsch wonders why Ribbert ever developed his unicentric origin of cancer since he abandoned it so soon for a multicentric origin and says:

In der Tat findet man auch in unmittelbarer oder etwas entfernterer Nachbarschaft von Haut–und Schleimhautkrebsen alle die Bilder, die Ribbert als 'beginnende Carcinome' gedeutet hat (p. 34).
. . . . Im übrigen scheint mir auch der grundsätzliche Unterschied zwischen dem Anerkenntnis des Wachstums von Carcinomen durch Zusammenfliessen multizentrischer Primärherde und der Vergrösserung durch Apposition ein sehr geringfügiger zu sein (p. 35).

Primary adenomas and adeno-carcinomas of the liver may also arise *multicentrically* and grow by *apposition*. We know this through the beautiful researches of Siegenbeek van Heukelom of Leiden (1894) confirmed independently by many persons: Witwicky (1899), v. Schmieden (1900), Cloin (1901), Catherine H. Travis (1902), H. Gideon Wells (1903), Weglin (1905), Polak-Daniels (1905), Horst Oertel (1905), Robert Muir (1908), Lindsay S. Milne (1909), Max Goldzieher (1910), Goldzieher and Bókay (1911), Saltikow *pro parte* (1912), and many others. Ribbert and his student Heussi (1898) denied this also, very emphatically, Heussi on the findings in one liver (28). Herxheimer also denied it (1906) on the findings in another liver (22).

The substance of Heussi's objections so far as derived from his studies are given below in the first paragraph, but much more important than any of his objective findings are his *theoretical objections* which are given in the last paragraph and to these I would call especial attention.

Aber immer ist eine Grenzlinie zwischen Geschwulst- und Leberzellen ganz genau zu sehen. . . . . Gebilde jedoch die noch zum Teil das Aussehen von Leberzellen, zum Teil schon dasjenige von Tumorzellen hätten und deswegen als Übergangsformen angesehen werden könnten, sind nirgends zu finden (p. 10).

But even he found three liver cells with double nuclei in the vicinity of a tumor nodule (p. 16). He also admits there are places where the boundary between tumor cells and liver cells was not sharp but attributes this to defective sections (p. 17).

Heussi also says of Siegenbeek van Heukelom:

Seine Bilder stimmen in allen Punkten mit den unsrigen überein, aber unsere Deutung ist eine andere. . . . . Unsere Auffassung ist zweifellos die einfacherer und natürlicher. Sie geht nicht auf eine völlig unerklärbare inficierende Eigenschaft der Tumorzellen zurück, sie verlangt von uns nicht, dass wir uns in Spekulationen darüber verlieren, wie denn die Leberzellen sich sollten in Tumorzellen umwandeln können (p. 25). . . . . Ein so völlig unerklärbarer, wir möchten sagen geheimnissvoller Vorgang wie er dadurch gegeben sein soll, dass die Geschwulstzellen fähig sind, die Leberzellen in Tumorzellen umzuwandeln, müsste in ganz unanfechtbarer Weise bewiesen werden, damit man sich entschliessen könnte, ihn zur Erklärung des Wachstums der Neubildung heranzuziehen (p. 26).

There are no illustrations and the whole Dissertation reads like a case of special pleading in a *parti pris*, nor must we forget in this connection that the work was student work done under Ribbert's all compelling direction.

Heukelom's plates (19) show exquisite transitions from liver cells to carcinoma cells and both the cells and the cell-nuclei are enlarged before they become tumor cells. The nuclei of the large cells are also conspicuously notched and cleft as in crown gall.

Frohmann the same year (1894) reached the same conclusion (20):

Ein grosser Teil der Leberzellen fällt sofort durch die ausserordentliche Grösse auf (p. 10).

    . . . . Was den Ausgangspunkt derselben [der Geschwulstknoten] betrifft, so lässt es sich mit Sicherheit fest stellen, dass sie ausschliesslich

aus den Leberzellen hervorgehen. Die unmittelbare Uebergang von letzteren in Geschwulstzellen ist in vielen Präparaten mit Leichtigkeit zu verfolgen (p. 12).

Von Schmieden (29) says:

Die wichtigsten hierher gehörigen Arbeiten stammen von Rokitansky, Wagner, Griesinger, Rindfleisch, Friedreich, Klob, Hoffmann, Eberth, Willigk, Wulff, Perls, Birch-Hirschfeld, Jungmann, Greenish, Pawlowski. . . . . Viele haben den Uebergang von Leberzellen in Geschwulst-zellen verfolgt. (p. 292). . . . aus diesen Riesenzellen wächst unmittelbar eine Brut hervor, die keine Leberzellen mehr sind, sondern Zellen des Tumors (p. 307).

. . . . Nirgends aber lässt sich jedenfalls die Entstehung von primären epithelialen Neubildung so genau und in so jungen Anfängen verfolgen, als bei den multiplen malignen Leber-Adenomen (p. 320).

Miss Travis (31) says:

The transition from these cords to the structure of the new growth is as follows. The cells in the surrounding cords become larger, their nuclei are also enlarged and take a deep haematoxylin stain and this widened atypical strand becomes still wider, comes to have several cells abreast, and passes thus gradually over into one of the cords definitely belonging to the tumor (cf. figs. 8 and 9). . . . . The manifold small nodules scattered through the liver are then not of metastatic origin, but are primary growths derived by a direct transformation from the liver cells (p. 111).

Wells (33) writes of his liver tumor:

. . . . The small secondary nodes around the primary one seem to be formed in this way by the starting up of malignant transformation in a lobule or group of lobules a little ways from the boundary of the primary growth. But there are no secondary nodules at any consider-able distance from the primary tumor. (p. 416).

. . . . This carcinoma does not seem to have grown by direct extension in the usual way, with new tumor cells crowding out the preëx-isting cells, but rather the existing cells themselves assume the power of proliferating in a malignant manner. (p. 416).

. . . . The independent malignant transformation of cells in the vicinity of a tumor must be of some significance. That it should be

observed particularly in the liver is, perhaps, due to the fact that the structure of the organ makes its detection simpler than it would be in other places—a similar extension of proliferation has been described in the genesis of carcinoma of the skin (Petersen) (pp. 416–417).

Oertel (34) says:

Verfolgen wir diesen Vorgang, so ergiebt sich deutlich, dass die Krebszellen aus den am meisten degenerierten Leberzellen hervorgehen. . . . . So ergiebt sich eine krebsige Umwandlung von Leberzellen innerhalb der Läppchen zunächst ohne *Kontinuitätstrennung* oder *Strukturveränderung* des Läppchens selbst (p. 508). . . . . Die krebsig entartenden Zellen sind nicht losgelöst, isoliert worden, vielmehr bilden noch nach der krebsigen Umwandelung und ehe ihre regellose Proliferation beginnt, ich betone es wieder, Teile der Leberbälkchen und Läppchen (pp. 510–511).

Goldzieher (37) says:

Wenn schon die in sämtlichen 14 Fällen wenigstens teilweise vorhandene morphologische Ähnlichkeit der Tumorzellen mit Leberzellen, ... einen genetischen Zusammenhang mit dem Leberparenchym wahrscheinlich macht, so wird dies durch eindeutige Ubergangsbilder wohl ganz bewiesen.

Sofanden sich weiderholt durch dass cirrhotisch vermehrte Bindegewebe abgegrenzte Leberläppchen, die zentral oder peripher eine direkte Umwandlung ihrer Zellen, in Tumorzellen morphologisch volkommen gleichende Zellen, zeigten.

Ebenso fand sich in der Nachbarschaft eines etwa haselnuszgroszen, akzidentell gefundenen Leberkrebses, ein kleines, aus wenigen Bälkchen hochgradig entdifferenzierter Zellen bestehendes Knötchen das, wie es die Serienschnitte lehrten, nirgends mit dem groszeren Herde zusammenhing, dagegen überall in die benachbarten Leberbälkchen überging. Ebenso kommunizierten seine Kapillaren mit den anstoszenden Leberkapillaren (pp. 334–335).

. . . . The origin of the hepatocellular carcinoma is probably multicentric . . . . auch durch *Apposition* vergröszen können, wie es besonders in einem meiner Fälle schön zu sehen war.

. . . . The assumption of an appositional growth in Carcinoma, not everywhere accepted, it is true, although as Lubarsch has pointed out, there is no essential difference between this growth and the multicentric growth—appears to be confirmed by these discoveries (p. 335).

In opposition to Ribbert, Goldzieher continues:

More important than the separation of liver cells from their fellows
appears to me to be that neverfailing phenomena of growth which was
always to be observed both in the vicinity of the tumor nodules and also
remote from them in the liver tissue.   I mean both *Hyperplasia*, with the
formation of small cells containing deep-staining nuclei, and *Hyper-
trophy*, that is, the production of large, very sharply-contoured [liver]
cells which often contain polymorphic nuclei and which also in some
cases lead to the formation of a surrounding benign adenoma of the liver
associated with which is a much larger tumor, everywhere of similar
origin but definitely carcinomatous (l.c. p. 336).

The next year (1911) Goldzieher and Bókay (38) expressed
themselves as follows respecting their primary liver carcinoma
No. 20.

Separated from the edge of the tumor by several rows of apparently
unchanged liver cells, there is a nodule composed of 6 cords of liver cells
the cells of which can be distinguished from the surrounding liver cells by
the more abundant chromatin contents and considerable polymorphism
of their nuclei, as well as by the weak basophil reaction of their proto-
plasm.   These cords, which are not composed of 1 or 2 cell-rows like
the surrounding liver-cell cords, but have 3 to 5 cell-rows are bordered by
capillaries and pass directly over into the surrounding liver trabeculae,
in which also scattering nuclei are visible that correspond exactly to the
tumor-cell nuclei.   This nodule is nowhere in connection with the larger
tumor, as shown by *serial sections*, and did not arise as the result of
capillary emboli.   The capillaries are everywhere free from tumor cells,
and between the cords in question, which resemble the tumor-cell
trabeculae, atrophied liver cells, flattened by pressure, are nowhere
to be found, such as are always to be seen even in the earliest stage of
embolic liver metastases.   Nothing remains therefore but to assume that
here is an *independent tumor* in the beginning of its development, with
direct conversion of liver cells into tumor cells (pp. 113–114).

Goldzieher and Bókay's observations are so interesting that
I have copied their figure 10 which shows in a small primary
cancer of the liver an enlargement of the liver cells on the edge of
the nodule as the first stage of their conversion into cancer cells

(fig. 2).   The margin of their tumor is surprisingly like my crown-gall margins.   The tumor consists, centrally, of a small area of deep-staining disoriented cancer cells, surrounded by pale-staining, big-nucleate hypertrophied liver cells, beyond which are the smaller normal liver cells.   There has been no invasion here but a change *in situ* from normal liver cells, through hypertrophied liver cells, into cells which are definitely carcinomatous.

Fig. 10. Vergr. Leitz. Obj. 6, Okul. 1.   *a* normales Lebergewebe. *b* geringe Hyperplasie der Leberzellbalken. *c* hypertrophische Leberzelle. *d* Karzinomtrabekeln.   (Fall 20.)
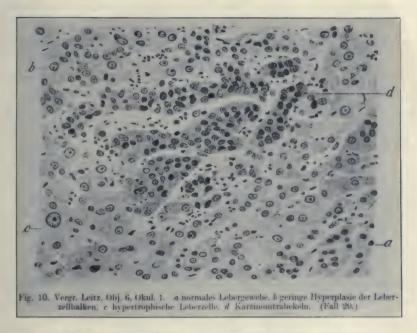
FIG. 2.   AFTER GOLDZIEHER AND BÓKAY.   SEE TEXT

In 1908, B. Fischer also stated in the most positive terms that he had found multicentric and appositional growth in a primary sarcoma of the liver arising from endothelium.   He writes (23) as follows:

F. berichtet über ein primär multiples Sarkom der Leber (45 jähr. Mann) welches von den Endothelien der Kapillaren ausging.   Diese seltene Beobachtung wird dadurch noch interessanter, dass sich überall in der Peripherie der Geschwulstknoten der kontinuierliche Uebergang des normalen Kapillarendothels in Geschwulstzellen nachweisen lässt.

Diese Geschwulst wächts also nicht allein aus sich heraus durch Vermeh-
rung der Geschwulstelemente, sondern vor allem auch durch fortschrei-
tende Umwandlung der normalen Gewebszellen in Tumorzellen. Der
Nachweis dieses Wachstums lässt sich hier einwand frei erbringen.

To return to crown gall, I am inclined to think that growth by
apposition is the common form of growth in this tumor on ac-
count of the fixed place of the cells composing plant tissues. Is
there then no invasion? Yes, but very often at least it is the
result of appositional growth in one direction only, end to end
growth, so to speak, leading to the production of an abnormal
strand between other tissues. Some, at least, of the tumor
strands appear to me to originate in this way. Possibly all do
so. See, for example, The structure and development of crown
gall. A plant cancer, Bulletin 253, B. P. I., U. S. Dept. Agri-
culture, 1912, plates 102, 103, where a tumor strand in the outer
cortex of a tobacco stem is figured. This strand, which begins
in a very shallow needle wound (crown-gall infection) at the
bottom of plate 103, ends diffusely in the large-celled cortex
parenchyma a little beyond the top of plate 102, as if it were
growing by conversion of cells rather than by an invasion *sensu
strictiore*, that is by wedging in between them (see also plates 17
and 18 of this paper). I cannot see that it makes any difference
in the final result whether a secondary tumor develops from a
migratory strand or from an appositional strand. They are
both invasions but arising in mechanically different ways cor-
responding to physically different cell-structures.

I figured some of my earlier findings of growth by apposition
in 1916 in this Journal, vol. i, no. 2, figs. 3, 4, 78, but the subject
is so interesting, and so new, that it is worth while to consider it
more in detail and especially to show good photomicrographs of
sections from characteristic tumors so that hereafter there may
be no doubt whatever as to its occurrence. This must be done
whether it points toward or away from human cancers.

The first stage of the conversion of cortex-cells into tumor
cells on the periphery of a growing tumor in tobacco cortex is
their enlargement (plate 1B). They become considerably

larger than normal cells (plate 1A), as may be seen also from an examination of the border tissue in the planar enlargements already referred to (plates 2A, 2B and 3A). Here the number of the cells has not increased materially yet the thickness of the cortex has nearly doubled. They are not only larger than the normal cells but also they are more inclined to divide, always by mitosis so far as I have observed. On the inner margin of this area of hypertrophied cells, next to the tumor, the cells divide, as a rule, much more rapidly, *that is, with unusual and very great rapidity*, acquiring at the same time a greater affinity for tumor stains than the remote normal cells or than the near-by enlarged cells. The daughter-cells in this region soon divide again and again, but some of the cells are still much larger than the tumor-cells, although staining more like them than they do like normal cortex-cells. They also have thinner cross walls than the normal cortex-cells *and no intercellular spaces*. The nuclei of these cells are also large, much larger than those of the completed tumor-cells. It is therefore often possible to see 4 or 8 or 16 or more of these cells enclosed by the stretched and thickened wall of the parent-cell (the original enlarged cortex-cell) as shown on plates 7, 11 and 16, and still more plainly in the cells of plates 4B and 5, where they look not unlike giant sarcina-cell packets, that is, the original or parent-cells are rounded and there are conspicuous intercellular spaces between them, as may be seen in many of the photomicrographs, whereas their daughter-cells are more or less angular and without intercellular spaces. In this respect they are like embryonic tissue and also like the fully formed tumor-tissue where, ordinarily, there are no spaces between the cells. This hasty cell-division which does not allow of cell-maturity proceeds in the region of the inner hypertrophy until groups of these cells are indistinguishable from cells in the body of the tumor, either in shape, disorientation, absence of intercellular spaces, reduction of cytoplasm, size of nucleus, behavior of nucleus, or affinity for stains, i.e., until they form lobes of the tumor. Sometimes a cortex-cell does not respond to the stimulus like its fellows and is therefore surrounded and buried in the tumor tissue, where it remains unchanged, or is

crushed, or tardily undergoes division. We may conceive of the stimulus as a chemical-physical one derived from the bacteria and acting either at a distance from them, i.e., on cells in which they are not present, or as due to a direct transfer of the bacteria from cell to cell, the adjacent walls having numerous very thin places (pits) through which such a transfer might easily take place by solution of the very thin membrane, or by its rupture due to pressure, in which latter case the chemical-physical stimulus would be confined to the parasitized cells or at least would not extend beyond their immediate vicinity. These pits are shown more or less indistinctly on various plates which were not focused for that purpose, and very plainly on the wall of a dividing cell in the middle of plate 1B. In cross-section the pit-walls are only one-eighth the ordinary thickness of the cell-wall. So far as the hyperplasia itself is concerned, as distinguished from the hypertrophy, I believe it is due to direct entrance of the bacteria into the rapidly multiplying cells whereas in the hypertrophied cells we may think that they have not yet entered or, if they have entered, have multiplied only in very small numbers so as not yet to cause a great hyperplasial stimulus which comes a little later when their by-products within the cell have increased and have had time to act, a period of a few hours or a few days only. It is possible also that the bacteria act only after they are dead. The narrowness of the appositional layer (0.5 mm. or less) indicates on the whole that it must be due to the direct movement of the bacteria from cell to cell rather than to the action of chemical products at a distance from them, otherwise how explain the slight diffusion of the stimulus? This peripheral layer in process of transformation is so characteristic that from an inspection of it crown-gall can be predicted. In a way, it suggests the large-celled, large-nucleate tissue often seen in early carcinomas and held to be typical (Cullen, l.c., fig. 230, p. 441.

The fully converted cells of crown galls may be either larger or smaller than the connective-tissue cells from which they have developed. For tumor cells larger than cells of the tissue from which they have developed see my Textbook (l.c.), figures

345 and 346. Much depends on how rapidly the tumor is growing. In the case of these young tumors developed in the soft tobacco-cortex, the cells have divided many times and so rapidly that they are very much smaller than the normal cortex-cells. None of them have had opportunity to become mature or even semi-mature. They also stain very differently. The tumor tissue treated with acid fuchsin and methyl green or with haematoxylin takes a deep stain while the normal cortex with proper washing and especially if counterstained retains scarcely any of the red or purple stain. It is the protoplasm, of course, which stains. The transition tissue stains like the tumor-tissue, but paler, it is, however, easily distinguished from it by the larger and variable size of its cells and their nuclei, and often also by the surrounding walls of the parent-cells which, however, become less evident as the divisions continue and the pressure increases. Its cells are readily distinguished from normal cells not only by the formation of thin cross-walls in various directions but also by the peculiar appearance of its cytoplasm (presence of numerous granules which are plainly much coarser than those of the normal protoplasm), and by the notched, cleft or mulberry shape of many of its nuclei. These latter phenomena as well as the phenomena of mitosis must be studied under high powers of the microscope and are not distinguishable on any of the photomicrographs here shown. For notched and cleft nuclei and abnormal mitosis see The structure and development of crown gall: A plant cancer, U. S. D. A., B. P. I. Bulletin 255, Washington, Government Printing Office, 1912, fig. 1 and plate 108. The enlarged cells beyond the active transition tissue, i.e., beyond the tissue plainly in very active disordered division (plates 1B, 5 and 12), possess intercellular spaces and stain like the normal cortex-cells, that is, very feebly, if the sections are not overstained and are properly washed. That they also are in process of division may be seen from the very thin cross-walls visible in many of them.

Often the appositional growth when it is very rapid so as to produce great pressure comes to an end suddenly by the crushing of remoter tissues (plates 3B, 4B, 8, 9, 10), and occasionally

it ends abruptly in some part of a tumor for no plain reason, in
which case the cells are flattened from the pressure but not crushed
(plate 4A).

The most striking thing perhaps in these tumors, aside from
their growth by apposition, is the rapidity of their growth and
the correspondingly small size and great immaturity of their
cells. Indeed, it is one of the most remarkable things I am ac-
quainted with in biology that a schizomycete should have such
power to change the behavior of a cell without destroying it
(see Ribbert's dogmatic counter statements, cited on page 8).
The tumor-cell is often only from $\frac{1}{50}$ to $\frac{1}{500}$ the size of the
cortex-cell from which it has developed (I am thinking here in
3 dimensions). Only the nucleus retains something like its for-
mer size and consequently nearly fills the cell leaving but scant
room for the greatly reduced cytoplasm. The nucleus of the
tumor-cell in these tobacco—cortex tumors is actually reduced
in size, *i.e.*, smaller than that of cells in the transition tissue but
is not reduced proportionately to the cytoplasm, nor anything
like proportionately. In the tumor, roughly speaking, $\frac{1}{5}$ to
$\frac{1}{15}$ of the cell-space on cross section is occupied by the nucleus.
In the normal cortex-cells, in the middle of the bark, the nucleus
occupies only $\frac{1}{50}$ to $\frac{1}{100}$ part of the whole area of the cross-section.
In general, I believe it is safe to say that there is 100 times as
much nuclear substance per cubic millimeter in the tumor-tissue
as in the normal cortex, out of which it has developed, and some-
times much more, but considerably less than in an equal volume
of embryonic tissue, developing roots for example as on plate
28. This fact of cell-immaturity, of greatly reduced cell-size
and of relatively greatly increased nucleoplasm, together with
absence of intercellular spaces and exhibition of great affinity for
protoplasmic stains, is characteristic and makes the tumor-tis-
sue somewhat resemble embryonic tissue, yet it is not embryonic
tissue. It does not grow as rapidly, its nuclear substance is
less abundant, its cytoplasm is more granular, its reaction to
stains is somewhat different (less deep and slightly different in
tone), its cells are less normally oriented, and finally it has
neither the persistent vigor nor the totipotent power of the

embryo. It cannot produce out of itself the whole plant or any organs of the plant but at most only a stroma of cells and vessels, and even this in many cases, and probably in all, arises out of the normal tissue *pari passu* with the round-about development of the tumor-cells, yet if totipotent cells or pluripotent cells are in its vicinity, or borne on its surface, or surrounded by it, the stimulus of the tumor sets them growing and then we may have a tumor full of fugitive shoots or roots or flower buds or tiny buried fragments of organs, that is, *an embryoma*. Root-anlage outside of a tumor, but near it, are very often set growing as shown on plate 28 at *R* and shoots behave in the same way. Any cancer-specialist who has worked much on embryonic tissues knows that they are quite unlike tumor-tissues, even when they occur exposed to them as tiny fragments in solid embryomas. This to my mind makes it unlikely that dislodged embryo-cells or misplaced tissues of any sort are the origin of malignant tumors. They may begin in such tissues—but why? I am quite of the opinion of those oncologists who maintain that the cancer-cell is a biologically changed cell; only in case of human and animal cancers we do not know what causes this change, whereas in crown galls we know that it is due to an intruding intra-cellular schizomycete.

Within the nucleus of the tumor-cells the nucleolus is often surrounded by a clear space which is very conspicuous, much more so than in normal resting nuclei but this may not be pathological. The nucleus also is often deeply and sometimes repeatedly notched or cleft even to complete division. This has been seen in the smallest tumor-cells in the center of these tumors but it occurs more especially in the actively dividing cells of the transition tissue on the margin of the tumors, that is, in the youngest part of the tumor. Here many nuclei are notched and cleft, and sometimes entirely divided, but whether this is wholly abnormal or follows the law of growth of tobacco-cortex under special conditions, *i.e.*, whether it can occur in the absence of tumors where growth is very rapid; and whether in the peripheral growth of these tumors the cell-division is wholly mitotic or both mitotic and amitotic must be left for further research.

I have seen cells in mitosis in the center of these tumors and spindle-figures in cells of various sizes on their periphery both in the actively changing part and in the hypertrophied cells outside of this part. So far as I have observed, however, mitotic figures are rare in all of these 15 tobacco-cortex tumors, *i.e.*, less than one per field of the microscope. This might mean either that growth was slowing down or only that the material was collected at the wrong time of day. All of the material was removed and fixed in the middle of the afternoon and it is assumed that most of the karyokinetic cell-divisions occur at night since we know that in many plants most of their growth occurs at night. From examinations of many sections of Paris daisy tumors made in my laboratory in 1911 from material fixed every hour throughout the night, as compared with sections of many tumors fixed in the daytime, it is plain that most of the cell-divisions in that tumor occur at night.

Not infrequently in the tumor-tissue and also in its vicinity two nuclei occur in a cell without any trace of a wall between them. On the periphery of a tobacco-cortex tumor in one of the larger cells I observed four well developed nuclei with no trace of any cross-walls even the most delicate separating them. Miss Lucia McCulloch and Miss Nellie A. Brown of my laboratory both observed and sketched the same thing in 1911 studying the night development of crown gall on the Paris daisy.

These tumors are so young that necrosis has not appeared in them anywhere, the only dead parts being certain crushed cells just beyond their borders, but when necrosis does occur in crown galls it begins centrally.

The two inoculated tobacco plants which furnished the material for this paper bear the numbers 1548 and 1549 and the various independent tumors on each are designated A, B, C, D, etc. All these tumors are of the same age (three weeks) except the small pith tumors, which I must think are secondary, and very young, probably not more than a few hours old, in case of the smaller ones (plates 25 and 26). All were produced by single needle pricks without hypodermic injection and consequently the primary infection was only in the cells wounded by the needle thrust.

All of the sections were stained in the same way, viz., several hours in 1 per cent Grübler's methyl green dissolved in distilled water, for the lignified tissue, which is stained blue; then, for a few minutes only, in 1 per cent Grübler's acid fuchsin dissolved in 70 per cent ethyl alcohol, for the tumor-tissue, which becomes red. After this they were washed and dehydrated by passing them very quickly through 85 per cent, 95 per cent, and absolute alcohol, after which they were passed through xylol and mounted in Canada balsam.

For further details the reader is referred to the plates. First an enlarged cross-section of the normal young cortex is given showing the type of cells wounded and what is assumed to have been the deepest wound inflicted. From this it will be seen that the deeper cells of the tobacco-cortex are larger than those near the surface, but this has made no difference in the result, many of the smallest tumor-cells having been derived from the large cells of the cortex rather than from the small ones. Then are given some low-power ($\times$ 20) planar views of sections from several of these tumors. After which follow, in medium enlargements ($\times$ 93 or $\times$ 205), photomicrographs of the margins and deep parts of various tumors cut in different planes. In one case (tumor 1548A) I have made a series of photomicrographs at different levels (see fig. 3) from near the center of the tumor on slide 7 to beyond its margin on slide 12. These are 20μ sections and the total distance traversed is 1660μ but in the fresh material of course, considerably more than that. The sections on slides 9 and 10 in this series are particularly instructive as may be seen from plates 6 to 8. Here the knife has passed parallel to the surface of the tumor in its extreme outer part or just beyond it (appositional layer), and there are striking exhibitions of transition tissue. In fact, on plate 7 the whole center of the plate shows normal cells in process of conversion into tumor-cells. On plate 8 may be seen the lower part of plate 7 (X corresponding to X) and here also the remoter hypertrophied layer, beyond which are normal cells. On slide 12 in this series we pass beyond the tumor but not entirely beyond its influence. For sections of the borders of other tumors passing through the

whole thickness of the appositional growth, *i.e.*, cut at right angles to plate 7, see especially plates 9 to 13.

In all or nearly all of these tumors the vascular cylinder has been split open by the growth of the tumor (plates 18 to 21) and the pith is in process of invasion. For the latter phenom-
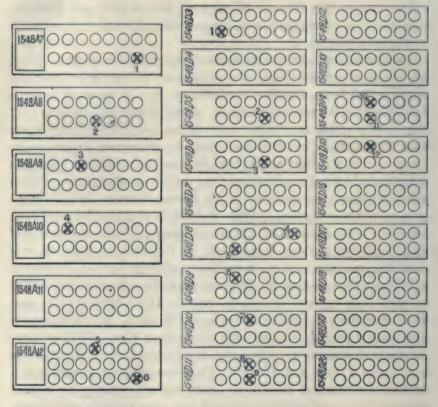

Fig. 3

Fig. 4

enon see plates 23 to 27, together with figure 4 which marks the location of the various sections from which the plates were made. In 1548D in the outer pith, beyond the advancing margin of the main tumor and close to the inner edge of the split wood, are various scattered small tumors (see plate 22 for orientation). I have not been able to connect back these small pith-tumors

definitely to the cortex-tumor by any strand of tumor-tissue and in this way they are like Krompecher's and Petersen's small tumors, arising independently in the vicinity of a mother carcinoma, as Petersen showed quite clearly by his wax-plate method. I have not applied this method but from a study of the serial sections I do not think it is possible that all may be connected by cell-bridges, even the tiniest. At least this is not evident. They appear to be metastases but they are not such in the true meaning of that term, and neither perhaps were Krompecher's or Petersen's, but if I am right in the explanation which follows as to their origin, then perhaps they might be designated *pseudometastases*.

Recently I have gone over all the serial sections again and can only conclude that while these small tumors are near the parent tumor and in tissue somewhat like regeneration tissue, developed in response, probably, to the wedging open of wood and pith by the continued deep growth of the primary tumor, they are not actually part and parcel of the mother tumor, but all would undoubtedly have fused with it a little later. In one instance a small tumor lies rather deep in the pith—a whole field of the microscope away from the inner wood, that is, about half a field farther in than the tumor on plate 24—and this appears to have developed from the proliferation of a few small pith-cells, making of it an irregular fine-celled, deep-staining strand between large pith cells. It contains only a few hundred small tumor-cells surrounded on all sides by pith cells. This tumor strand begins on slide 16 and ends on slide 23, and I have not been able to connect it with any of the discrete small tumors already mentioned or with the primary tumor. Here also I saw conspicuously notched and cleft nuclei, both in the tumor tissue and in the pith cells immediately surrounding it (slide 19). A similar isolated strand containing a few hundred cells only is shown on plate 27. This begins on slide 12 and ends on slide 14. Four of the smallest crown-gall tumors I have ever seen are shown on plates 25 and 26. They represent relatively few cell-divisions (disoriented, large-nucleate and deep-staining, be it observed) and cannot be more than a few hours old.

We may suppose these small pith tumors, if they are really secondary, originated in this way; that during the tearing open of wood and pith, resulting from the rapid growth of the primary tumor, certain of its infected cells were crushed liberating into the wounded wet area some of the motile rods of the parasite which then made their way through intercellular spaces or fissures into a few of the torn pith cells along the line of the rupture, converting these cells into a dozen or more new centers of tumor growth.

For further consideration see the plates and the accompanying descriptions.

In many of these sections, as in some of those from the Paris daisy tumors (see An introduction to bacterial diseases of plants, W. B. Saunders Company, Philadelphia and London, figs. 353 and 354), there are in the same tumor two types of tumor-cells, a spindle-cell originating from cambium and an ordinary round-cell of variable size derived from the cortex. The spindle-celled tumor tissue occurs not only in the deep parts of the tumor, near the ordinary cambium, but also in the outer parts of the tumor as if derived from incipient cork cambium, but I have not been able to trace the origin of the latter very clearly.

What resemblance, if any, the phenomena here described may have to peripheral growth in animal and human cancer must be left for the oncologists to determine. As we have seen from the statements cited in the first part of this paper, students of cancer are poles apart in their views as to how primary cancer grows in tissues of its own type, but it will be observed that there is a wide difference in the value of the two kinds of statements since the one kind are affirmations based on observations while the other are denials based on inability to see. I do not take Ribbert's statements seriously, because I do not regard Ribbert and his school as biologists at all but only as morbid anatomists and the solution of the cancer problem must come I think, from experimental biologists. In this connection it might be well to remember that when a man approaches a problem with a preconceived notion he is often as blind as a bat to the plainest phenomena. Every experimenter knows this from his own

observation and not infrequently from his own experience. One of the important things to be settled, it would seem is whether anything like what I have here described occurs in human cancer. Hauser, Hansemann, *et al.*, say it does; Ribbert, Borst, *et al.*, say it does not. If it does occur, then it is one of the strongest evidences pointing toward parasitism and it does not need to occur always to be important, nor need it be in any way confused with invasion, which is the entrance of the cancer cells into tissues of other types where in general no claim is made that there is any growth by apposition (see views of Hansemann and others cited in this paper).

SUMMARY

In addition then to (1) the absence of any capsule and conversion of cortex-cells into tumor-tissue by contact (growth by *apposition*), something easily to be understood in this tumor because it is due to an intracellular schizomycete and the adjacent cellulose walls of the cortex-cells, ray-cells and pith-cells are numerously pitted and are fundamentally all one type of tissue, the photomicrographs show a number of other interesting features; (2) the frequent limitation of the appositional growth through the crushing of remoter cells of the cortex; (3) the limitation of peripheral growth on one side or lobe of a tumor for no apparent reason while it continues on the other side or lobes; (4) the penetration of the tumor by way of the medullary ray across the phloem, cambium and the woody cylinders which are split apart; (5) the formation in some cases of independent small tumors (pseudometastases) in the pith near the primary tumor although the inoculations were restricted to the cortex; (6) the downward invasion of a medullary ray (beginning of a tumor-strand) in the wood as shown on plate 17; (7) the small size and immaturity of the tumor-cells in comparison with the size and age of the mother-cells and their great affinity for tumor-stains, as may be seen by the contrast in color of the normal and abnormal parts on the photomicrographs, the deeply stained parts of the sections having photographed dark and the pale parts light; (8) the enormous multiplication of cells considering that the tumors

were produced by single infected needle-pricks and that the whole period of growth was only three weeks; (9) the absence of any intercellular spaces in the tumor tissue or in the rapidly dividing transition tissue; (10) the distinct enlargement of the cortex-cells before their conversion into tumor-cells, which leads to a thickening of the cortex around the tumor, as shown on the plates already referred to, a sort of cushion being formed of which the tumor is the center; (11) the tendency of the nuclei in the transition tissue to be large and to be variously notched, cleft, lobed, or mulberry-shaped and the occasional occurrence of 2 to 4 nuclei in the cell; (12) the big border around the nucleoli, perhaps only indication of rapid growth; (13) numerous faint-staining abnormal granules in the cytoplasm of the transition tissue and of the tumor tissue as seen under high powers; (14) the fact that in young plants (those less than half grown) almost any cortex-cell is capable of further and repeated division, especially under a tumor-stimulus, whereas results on old tissues tend to confirm Bard's view that the reproductive capacity of old cells is zero; (15) development of roots under and near the tumors as a result of the tumor stimulus; (16) experimental disproof of Ribbert's dictum that parasites cannot change the form of cells or cause them to proliferate. Schmieden's words respecting his liver tumors describe the hypertrophy on the margin of these crown galls exactly: aus diesen Riesenzellen wächst unmittelbar eine Brut hervor, die keine Leber [Cortex]-zellen mehr sind, sondern Zellen des Tumors.

RECAPITULATION

The collateral enlargement on which the tumor rests, is both a hypertrophy and a hyperplasia. It has three stages of development; it is first a hypertrophy, then an accessory hyperplasia, and finally on its inner face it becomes part of the tumor itself.

[All the photomicrographs here shown were exposed and developed by the writer, but the prints for the half-tones were made by James F. Brewer. The serial sections were embedded, cut and stained mostly by Helen Fox, but a few by Lucia Mc-

Culloch. The photomicrographs were made on Cramer's Iso slow plates, except plate 20 which was made on a Seed's no. 30 Gilt Edge plate. All were made without use of color screens.]

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    [The part relating to primary cancer of the liver deals with 6 cases.]

    "In these six cases the origin of the tumor from liver cells is undoubted. In all of them the cells of the growth are in places closely similar to liver cells, and in five of them direct continuity with the liver columns can be traced at places, the appearance indicating multiple foci of origin (p. 299).

    The origin of the growth in all cases is from the liver cells. . . . . The cancer takes origin in multiple independent foci. In many of the small nodules direct continuity with the liver columns can be seen. The conclusion seems inevitable, that these young nodules are not secondary but are fresh foci of growth. (Summary, p. 302.)

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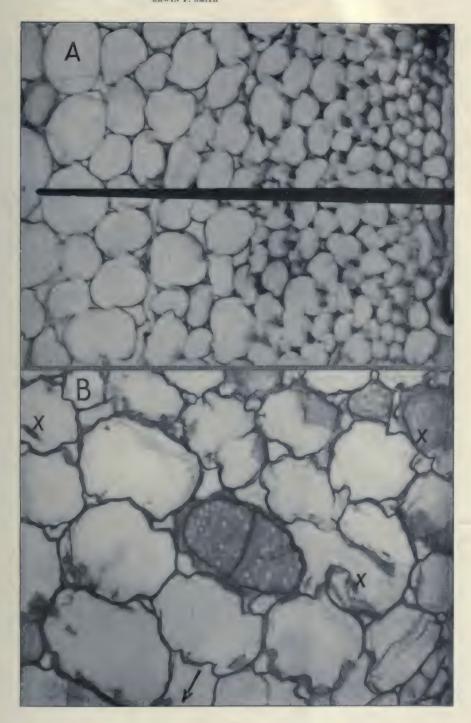
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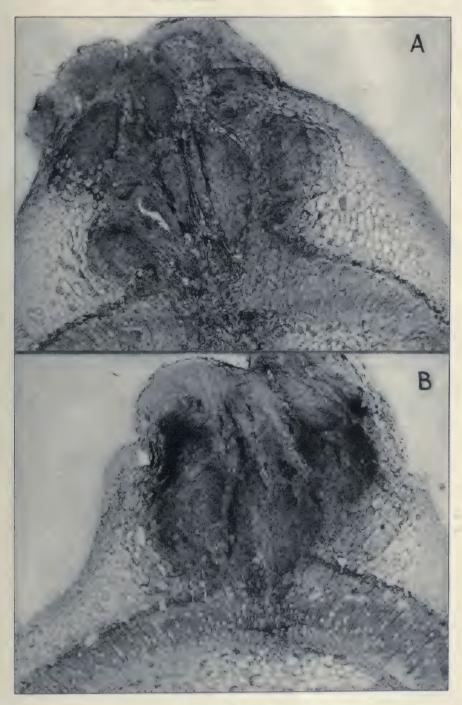
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PLATE 1

1A.   Young (stained) tobacco cortex in plant 1548 near tumor A, but not influenced by it.   Epidermis at right.   All of the cortex is included except 2 or 3 rows of cells at the left.   The type of inoculation (needle-prick) is also indicated and the results show that many of these cells were young enough to proliferate. At this level very few nuclei are visible, but more were visible at a slightly different level.   Very few of the cells are in process of division.   Slide 1548 A 4, lower row, sixth section from left.   8 mm. 4. c.   Bellows 40.   × 205.
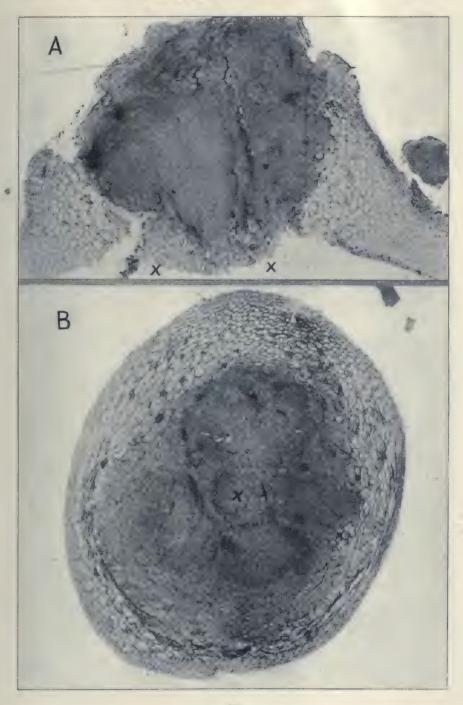
1B.   Slide 1548 D 5, lower row, fourth section from the left showing in cross-section hypertrophied cells bordering on the tumor which is one field of the microscope (8 mm. 4 oc.) away from the center of the photomicrograph in the direction of the arrow.   Several of the cells show pits (thin places) on their walls.   The wrinkling of the walls is due to shrinkage during fixation.   Nuclei out of focus are shown at X, X, and the intercellular spaces are very distinct.   The bottom of this plate joins on to the top of plate 5.   × 205.

PLATE 2

2A. Cross-section of tumor 1548 D showing various lobes resulting from appositional growth. Pith at extreme bottom, right side, vascular ring invaded and ruptured with beginnings of tumors in the outer pith; cortex thickened on either side of the tumor and its cells enlarged. Growth by apposition was still proceeding. Slide 10, top row, third section from the left. Planar 35 mm. Bellows at 58. × 20.

2B. Cross-section of tumor 1549 D. Cortex above and pith at bottom. Shows splitting of the vascular ring (center) and thickening of the cortex near the tumor with enlargement of its cells. Growth by apposition is present on both sides. Slide 16 (which passes through about the middle of the tumor), top row, first section at left. Planar 35 mm. Bellows at 58. × 20.
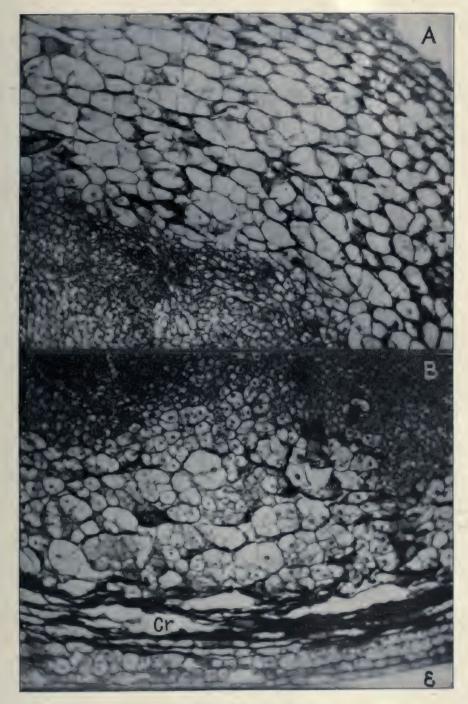
3A. Cross-section of tumor 1549 A. Unfortunately most of the wood (X, X) was removed before it was embedded. Vascular cylinder invaded and split open, cortex thickened and its cells enlarged on either side of the tumor. Slide 7, top row, last section at left. Planar 35 mm. Bellows at 58. $\times$ 20.

3B. Tangential section of tumor 1549 I (tangential to the stem). Lobate tumor in center surrounded by cortex. Needle prick at X. Growth of tumor stationary above or nearly so; marked appositional growth in progress on the sides and below. There is also crushing of tissues two-thirds of the way around the tumor where the growth-pressure has been greatest (for details see plate 4). Slide 12, top row, second section from the left. Planar 35 mm. Bellows at 58. $\times$ 20.
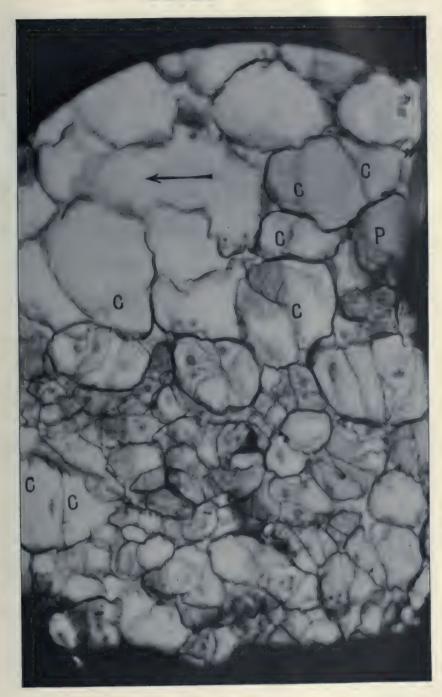
4A. Same tumor as in plate 4B (1549 I), but from the other side. The cut is tangential to the stem. Here growth has ceased or nearly ceased, the tissue is not crushed, and the tumor-tissue abuts on pressure-flattened and slowly dividing but otherwise nearly normal cortex, *i.e.*, there is not much evidence of conversion by apposition here, but there is a little (at the bottom right and in the middle left part), and of course there may have been much more earlier and might have been later. Slide 11, upper row, last section at right. 16 mm. 4 oc. Bellows at 40. × 93. For orientation see plate 3B.

4B. Tangential section of tumor 1549 I. Lobes of tumor tissue at top, transition cells of various sizes in middle with crushed tissue on the outer border (Cr) beyond which at the right are 3 rows of unchanged small cortex cells and the epidermis (e). Notice the dyad and tetrad groups of cells in the transition tissue. Slide 12, top row, 3d section from the left. 16 mm. 4 oc. Bellows at 40. × 93.

PLATE 5

Tumor 1548 D, slide 5, lower row, 4th section from the left, showing stretched cells at the top, some of which (cc) are dividing and below these more actively dividing transition tissue bordering on the tumor. In the middle are 3 rapidly dividing cells in a row, with the wall of the parent cell well preserved; nuclei are visible in these cells and half a dozen faint cross-walls. Below at the left also is a stretched cell with two delicate cross-walls, C, C. Pits on wall of a cell at P. The surface of the stem is in the direction of the arrow. 8 mm. 4 oc. Bellows at 40. × 205.

PLATE 6

Cross-section of tumor 1548 A, made close to its surface, especially in the central and upper part of the figure. Wood and phloem in upper left corner. Tumor cells in center and at right. The section cuts deeper into one of the tumor lobes in the lower right corner than elsewhere. Transition tissue at left from top to bottom, *i.e.*, deep cortex-cells being converted into tumor-cells. Especial attention is called to the middle of this figure, for comparison with the next plate.

The black dots are deep staining nuclei. The center of this plate covers exactly the center of plate 7 but is 300 μ nearer the great mass of the tumor, *i.e.*, while tumor-cells occur in the center of this section, in the same region on slide 1548 A 10 (see plate 7), we have only transition tissue, in other words, there we are beyond the tumor proper (except one lobe of it in the lower right corner) but in cortex-tissue which is becoming tumor tissue. For orientation consult figure 3, sub 3. Slide 9, top row, 3d section from left. 16 mm. 4 oc. Bellows at 40. $\times$ 93. The center of this tumor which is found on 1548 A, slide 7 (Fig. 3, sub 1) shows invasion of the wood.

PLATE 7

Cross-section of tumor 1548 A, but 300 μ (fifteen 20 μ sections) farther out than plate 6. For orientation see figure 3, sub 4. Vascular cylinder in the upper left corner, a lobe of tumor-tissue in the lower right corner. All the tissue between is *cortical tissue becoming tumor tissue* and may be compared with plate 1A, making some allowance for difference in magnification. For the same section about one-half field at the left of this field, see plate 8. Tumor 1548 A, slide 10, top row, second section from left. 16 mm. 4 oc. Bellows at 40. $\times$ 93.
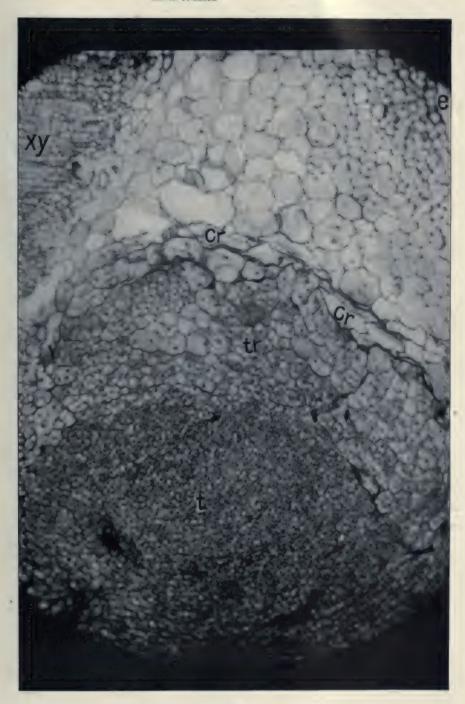
63

Cross-section of tumor 1548 A 10. Same section and same orientation as plate 7, but a little farther to the left, X in this plate corresponding to X in plate 7. Normal cortex at left, enlarged cells in the middle, rapidly dividing transition tissue on the right (above) then crushed tissue with tumor tissue below, which is black because the plate was under-exposed for this deeply red stained part in order to bring out more clearly the cells above it. Torn vascular cylinder at the top. 16 mm. 4 oc. Bellows at 40. × 93.

Cross-section of tumor 1549 A. Tumor tissue (t) at bottom, rapidly dividing transition tissue (*tr*) in middle with 3 or 4 different sizes of cells, then stretched and crushed tissue (*Cr*) and beyond this, enlarged cortex-cells, those at the right in division. Contrast size of nuclei in *t* and *tr*. Epidermis at *E*. Slide 10, middle row, 4th section from the left. 16 mm. 4 oc. Bellows at 40. × 93.
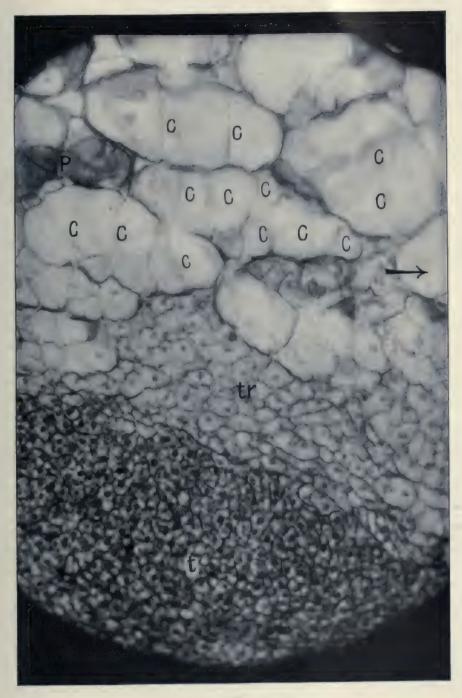
PLATE 10

Cross-section of tumor 1549 C. At bottom, tumor-tissue (*t*); in the middle transition cells (*tr*) of various sizes bordered by crushed cells (*cr*); at the top, cortex on the right and vascular tissue (*xy*) on the left. Epidermis at *E*. Slide 24, lower row next to last section on the right. (For opposite margin of this tumor see plate 11). 16 mm. 4 oc. Bellows at 40. × 93.
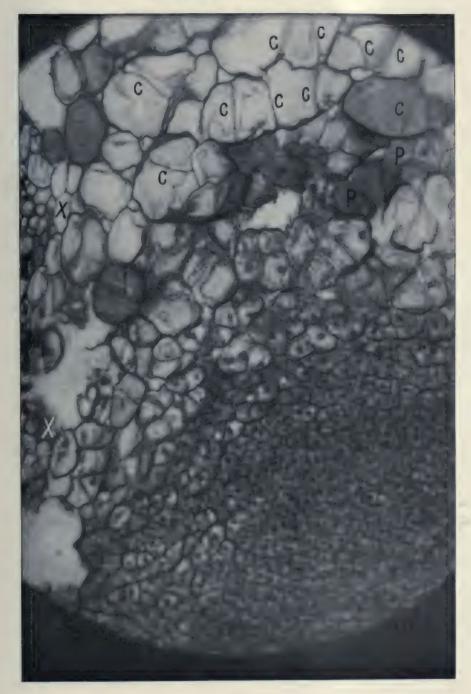
Same tumor (1549 C) and same section as on plate 10, but from the other side. Tumor cells (t) at the top, transition tissue in the middle (from A to B), unchanged cortex at the bottom. Surface at S and, under this, spindle-cells mentioned in the text. In places in the transition tissue the stretched outline of the original cells can be made out owing to the enclosing older thicker cell walls, the daughter cells being angular and having no intercellular spaces. The actual diameter of the transition tissue in this section is 1/2 mm. and as in the preceding there is a very good gradation from transition tissue into tumor tissue. 16 mm. 4 oc. Bellows at 40. $\times$ 93.

PLATE 12
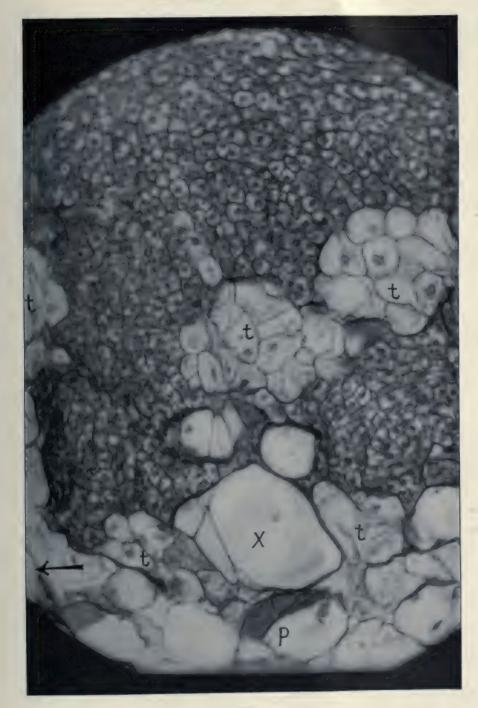
Cross-section of tumor 1549 D. Tumor tissue at bottom (t), rapidly divid-
ing transition tissue in middle (tr), at the top stretched cortex cells dividing
more slowly. At C, C, are delicate cross-walls in the stretched cells. The round
dark bodies are nuclei. Pits in the cell wall at P. Observe intercellular spaces
between the stretched cells and absence of them in the tumor tissue and in the
rapidly dividing transition tissue. Surface of stem in direction of arrow. Only
about 1/20 of the tumor area is here shown. Tumor due to a single needle-prick,
time 3 weeks. Slide 24, top row, 4th section from the left. 8 mm. 4 oc. Bellows
at 40. $\times$ 205.
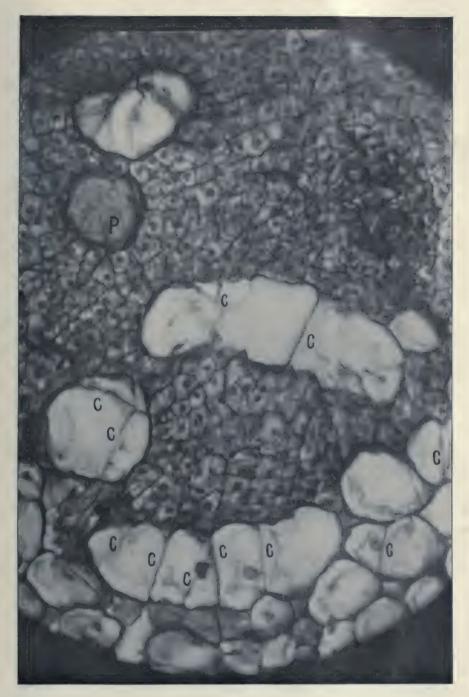
PLATE 14

Cross-section of margin of tumor 1549 D, showing tumor-tissue with very conspicuous nuclei, transition tissue at t, t, where the nuclei are largest and a stretched cell at X. Pits on cell wall at P. Surface in direction of the arrow. Slide 21, upper row, first section at left. 8 mm. 4 oc. Bellows at 40. $\times$ 205.

PLATE 15

Cross-section of the margin of a lobe of the tumor 1549 D, showing stretched cells some of which are buried in the tumor tissue. These stretched cells have formed delicate cross-walls at C, C, and the nucleus is visible in a number of the segments. At P pits on the wall of a buried cell. On this side of the tumor in this section growth is slowing down but on the other side there was rapidly dividing transition tissue, and on this side also at a different level, as may be seen on plate 2B. Tumor 1549 D, slide 25, top row, 5th sction from the left. 8 mm. 4 oc. Bellows at 40. × 205.

PLATE 16
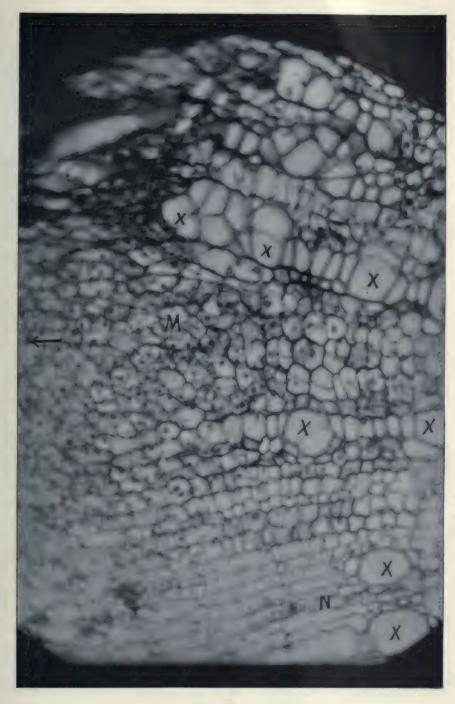
Undulate margin of a lobe of tumor 1549 D on slide 22, bottom row, second section from the left. The section, cut tangentially, shows lobules of tumor tissue mingled with transition tissue. At the top there are stretched cells. In the center the outlines of some of the original stretched cells are still visible although they have divided several times. Under P pits are visible in the wall of a cell. The arrow points to the surface. The bulk of the tumor is in direction of the two arrows. This section is near the surface of one of its lobes. 8 mm. 4 oc. Bellows at 40. $\times$ 205.

PLATE 17

Tangential section of deeper part of tumor 1549 I, that is, a section from slide 17 (top row, 3d section from the left), passing through the wood. Middle, at the top, tumor tissue breaking across the vascular ring; under this the tumor stimulus is propagating downward in a medullary ray (*M*), *i.e.*, here is the beginning of a tumor strand. The two medullary rays at the right of this are also not exactly normal, *i.e.*, their cells are beginning to divide. The tracheids *tr*, *tr*, are also disturbed, that is a tumor stroma is beginning to form. 16 mm. 4 oc. Bellows at 40. × 93.

PLATE 18

Photomicrograph from tumor 1549 D, slide 13, top row, third section from left, showing a wide medullary ray (M) due to appostional invasion of the tumor. Tracheids in cross-section at X X. Normal medullary ray at N. Most of the others are more or less abnormal. This is a much earlier stage of invasion than that shown on plates 19 and 21. The surface of the stem and the bulk of the tumor are in the direction of the arrow. The enormous number of pits on the tangential walls of the ray cells would seem to greatly favor the inward movement of the tumor impulse. 8 mm. 4 oc. Bellows at 40. × 205.

PLATE 19

Cross-section of tumor 1548 A, slide 7, lower row, last section but one at right (see fig. 3, sub 1) showing a wedge of tumor tissue separating the vascular ring. The wedge is wider and pushes in farther on other sections. A vertical section of this wedge along the line indicated (X—X) or rather somewhat deeper would have resembled the upper part of plate 17. 16 mm. 4 oc. Bellows at 40. × 93.

PLATE 20
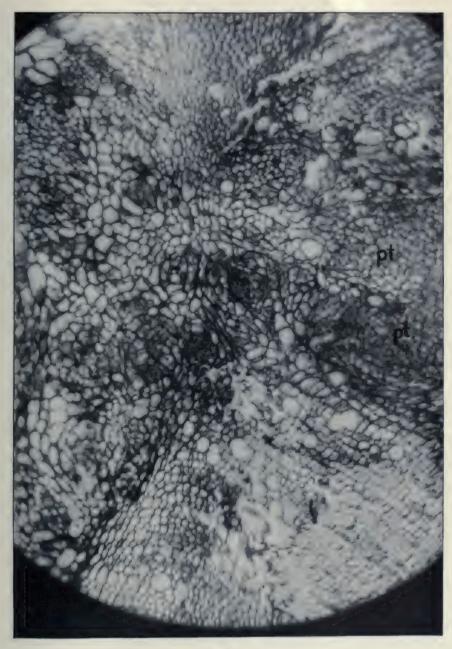
Cross-section of tumor 1549 F. slide 9, lower row, last section at right. Field showing fan-shaped aggregation of vessels (tracheids) at either side of a mass of fine-celled tumor tissue in what may be assumed to be the region of the needle-prick. These tracheids are part of the stroma but I assume them not to be direct outgrowths of the tumor-cells but to have been laid down out of normal tissues early in the development of the tumor, that is, soon after the needle-prick was made. The middle outer surface of this tumor (region of the needle-prick) is just beyond X. Several other tumor lobes are visible $(t. t, t)$. The photograph does not give the contrast of the stains because the tumor cells are red and the tracheids a bright blue. 16 mm. 4 oc. Bellows at 40. $\times$ 93.'

PLATE 21
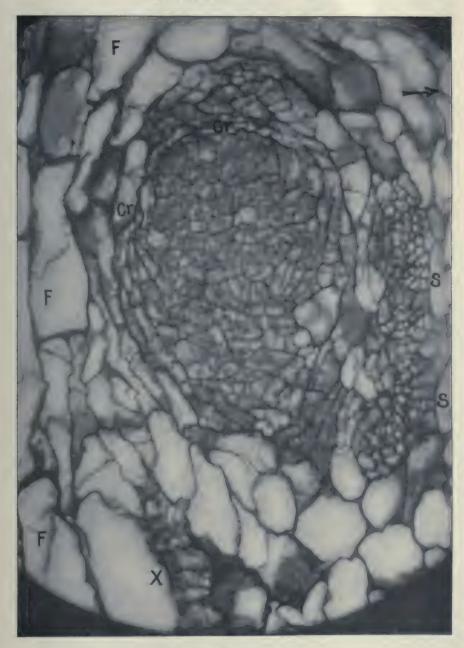
Cross-section of inner part of tumor 1549 G, showing the tumor-cells wedging apart the wood-cylinder. The cambium line is at C, C, and the entire width of the tumor wedge in the cambium region is about 1 mm. Slide 4, lower row, last section at right, 16 mm. 4 oc. Bellows at 40. $\times$ 93.

PLATE 22
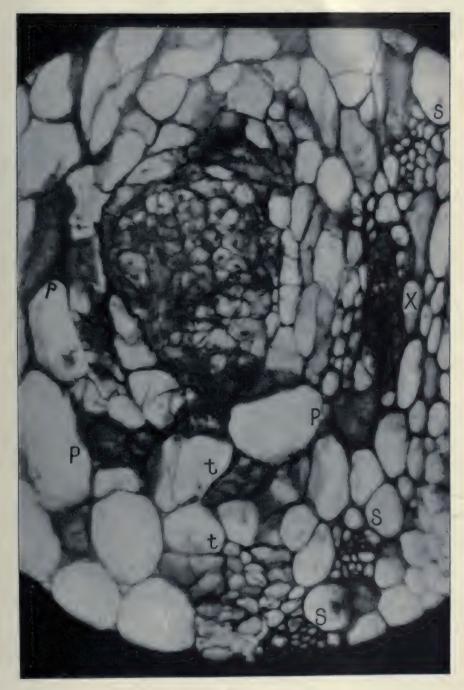
Cross-section of tumor 1548 D at its inner edge (right side of plate) showing tumor wedge in the wood in the middle of the right side (pt) and several incipient discrete little tumors in the outer pith (middle and left side). Slide 9, top row, 2d section from the left. 16 mm. 4 oc. Bellows at 40. × 93.

PLATE 23

Cross-section of tumor 1548 D, *i.e.*, the same subject as plate 22, but more highly magnified and from slide 6, lower row, 4th section from the left, to show a small nodule of tumor tissue in the outer pith. At the top and left side of this tumor there are flattened cells (*F, F, F*) and crushed cells (Cr). Tumor-cells are visible also at *X*. The fine-celled tissues at *S, S*, are groups of the inner sieve tubes in cross-section. The wood-cylinder is in the direction of the arrow. The main tumor shown here begins on slide 3 and runs out on slide 9, and I was not able to connect it with the primary tumor by any tumor-strand or tumor-cells, but it is not far from other smaller tumors which are nearer the main invading mass; nor was I able to connect the strand-like tumor at *X* either with this tumor or with the primary tumor. It begins on slide 6 and runs out on slide 8, but some other small tumors appear on sections of that slide above and at the right of this area (See plate 22). This is the only instance in which I observed tumor tissue growing beyond the crushed tissue (above the upper *Cr*). 8 mm. 4 oc. Bellows at 40. × 205.

PLATE 24

Same primary tumor (1548 D) as plate 23 and from same general locality, that is, the outer pith, but from slide 15, upper row, third section from the left. *i.e.*, $20.0\,\mu$ (101 twenty μ sections) intervene between this section and the one from slide 6 (plate 23). The whole 2 mm. thick region is more or less cancerous, *i.e.*, several tiny tumors are scattered about in the pith, and between these and the body of the primary cortex-tumor there are others in the repair tissue in the region of the inner wood along the line of the primary invasion but they are not connected with each other or with the primary tumor as far as I can determine. At the right, at S, S, S. and X, as on plate 23, are groups of sieve tubes in cross-section. Below the principal nodule are smaller groups of tumor cells (at t, t) and there appear to be some deep-staining tumor cells mingled with the sieve tubes at X, but these are all confined to a few sections. The large cells are pith-cells unchanged below especially at the left but flattened above by pressure and dividing. At $P\ P$, pits are visible on cell-walls. This tumor (the larger one) begins on slide 14 and ends on slide 15 (there are twelve $20\,\mu$ sections on each slide in this series) and I cannot connect it back to the primary tumor by means of any tumor-strand, although like the preceding tumor (plate 23) it is not far from the main tumor-mass. In this connection it should be remembered that *Bact. tumefaciens* is a motile organism and that it might perhaps reach and enter particular cells just beyond the margin of the primary tumor through rupture of tissues as indicated in the text, in which case discrete small tumors (pseudometastases) would be produced and a chain of tumor-cells would not be developed. 8 mm. 4 oc. Bellows at 40. $\times$ 205.

PLATE 25

Tumor 1548 D, slide 8, lower row, 2d section from the left. In the center are two small tumors (t,t) in the ruptured area close to the inner wood (which is on the right side in cross-section); tumor tissue also occurs at t'; sieve tubes at S, S'. The sieve bundle S', and the tracheids tr, are on the margin of the rupture and are undisturbed. The sieve tubes at S are pushed in. At st are vessels separated from their fellows (x, x, x, tr). Beyond the arrow (about 1–1/2 inches at this magnification) lies the tumor shown on plate 23. Twisted (disturbed) tracheids of the inner wood at XXX. These tumors begin on slide 8, and end on slide 9. Two others in the same relative position but from slide 14 are shown on the next plate. 8 mm. 4 oc. Bellows at 40. × 205.
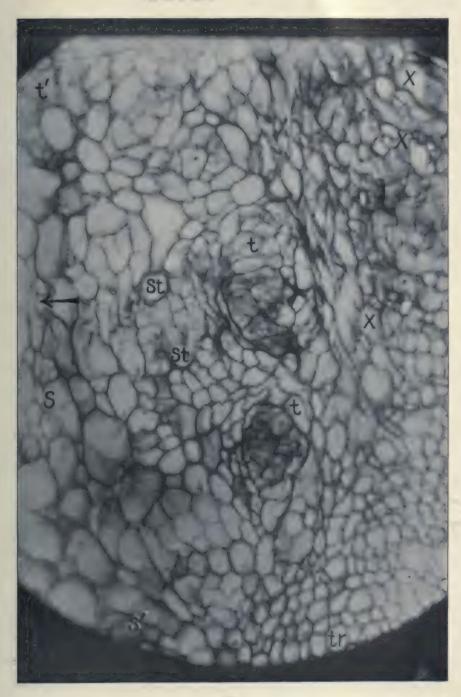
PLATE 26

Tumor 1548 D, slide 14, lower row, 3d section from the left. Same orientation as plate 25. In the center in the ruptured area are two small tumors (*t*, *t*) at the junction of wood and pith. These tumors begin on slide 13 and end on slide 14. At *X* in the inner wood is the advancing margin of the primary tumor. At *st* are separated vessels; at *tr* the vessels are in place. A vessel just beyond the upper *st* (out of this field) is crushed by pressure. 8 mm. 4 oc. Bellows at 40. × 205.

PLATE 27

Tumor 1548 D, slide 14, top row, 3d section from the left. Deep staining, strand-like small tumor in pith, just above the central tumor of plate 24. It begins on slide 12, and ends on slide 14. Another group of tumor cells at t'. The surrounding pith cells are under pressure (flattened) and are beginning to divide. Inner sieve tube region at S, S, S. 8 mm. 40 c. Bellows at 40. $\times$ 025. The half-tone does not keep the contrast of the photograph or of the stained section.
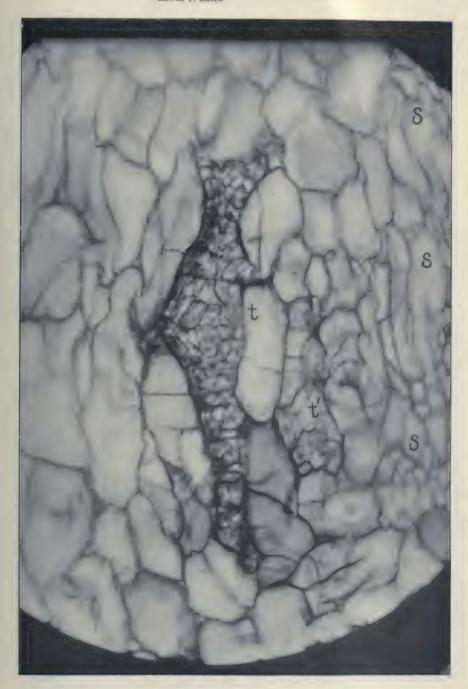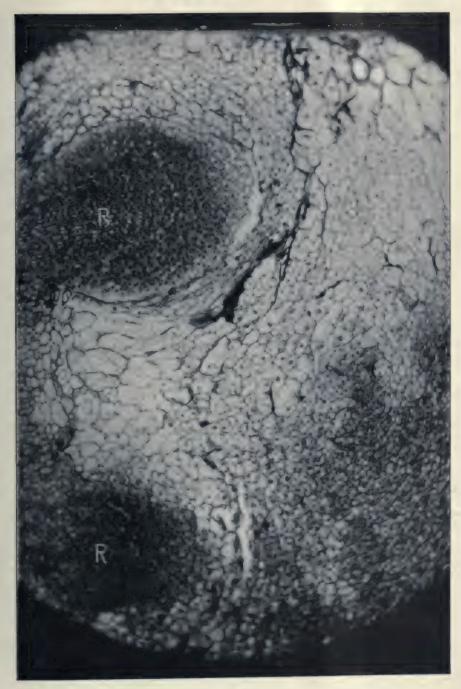
Cross-section of tumor 1549 E. Deep tumor tissue (lower right) and transition tissue in middle and on the upper right; outer part of the vascular ring on the extreme left and arising from it, at *R, R*, two incipient roots stimulated into development by the growth of the tumor. Slide 9, top row, 2d section from the left. 16 mm. 4 oc. Bellows at 40. × 93.

BIOLOGICAL EVIDENCE FOR THE INHERITABILITY OF CANCER IN MAN[1]

STUDIES IN THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

Eighteenth Report

MAUD SLYE

From the Cancer Laboratory of the Otho S. A. Sprague Memorial Institute and the University of Chicago

The biologic problem of the nature and inheritability of spontaneous cancer has been under study in this laboratory for the past twelve years and for ten years the results of that study have been in published form. During this time an increasingly broader and deeper mass of facts, always perfectly consistent, has steadily been obtained. These facts have been presented before scientific societies year by year, and have been published with masses of exact data (1–16).

One of the facts demonstrated in these studies is the inheritability of the tendency to spontaneous cancer, with its strong evidence against the probability of cancer being a specific germ disease.

The more superficial aspect of this demonstration, viz., its practical meaning to the human race, is the phase of the work which has mainly impressed the pathological and medical world. The more profound and biologic aspect of the demonstration has, for the most part, failed to make an impression, and there is a rather wide-spread medical opinion today, backed by some published pathological opinion represented, for example, by

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[1] Presented before the National Academy of Science, Chicago, November 15, 1921, and before the American Society for Cancer Research, Washington, D. C., May 1, 1922.

Ewing in his *Neoplastic Disease*, that a demonstration of the inheritability of cancer in mice (which is now quite generally conceded) has no bearing upon the question of the inheritability of cancer in man.

Let us for example examine the section on heredity in the second edition of Ewing's *Neoplastic Diseases* (17). This section scarcely permits of constructive criticism, for in one paragraph the author admits that the cancer tendency is inherited in certain human families, and in the next he vigorously states that "this fact in no way justifies the assumption that hereditary influences prevail for cancer in general." This is as if he should state that although black hair is hereditary in some families of negroes, it is not inheritable in the rest of the negro race where it also uniformly occurs.

Again, referring to laboratory studies in the inheritability of animal cancer, Ewing states that Tyzzer has demonstrated the hereditary theory for one family of mice, but he nowhere admits the general application of the laws of heredity, as, for example, to the rest of Tyzzer's cases or to the 4000 cases in the Slye stock, where cancer also uniformly occurs in exact accordance with the laws of heredity. This is as if he should say that albinism is inheritable in one family of the Tyzzer stock of mice, but that there is no justification for supposing that albinism is inheritable in the rest of the Tyzzer stock; or in the Slye stock where it has uniformly occurred in about 20,000 cases in exact accordance with the laws of heredity.

Again, on page 107 Ewing states that "human statistics of cancer heredity are worthless," but in the next paragraph he states that a pronounced hereditary predisposition to the disease does exist in the human families mentioned by Warren, Broca, etc.; and he finally closes the discussion by stating that "we must go into the field of human statistics" (which he has above pronounced worthless) "in order to determine by observation just how far any hereditary influence proves effective in the causation of tumor."

On account of such published opinion, totally inconsistent as it is, and the prevalence of similar ideas in many sections of the

medical profession, it has seemed advisable at this time to present the more fundamental aspect of the matter, and to point out what is the bearing upon the human cancer problem of these studies in the inheritability of animal cancer.

Under every smallest instance of behavior in the organic world which has ever been studied, there is biologic law. It is contrary to every biologic analogy to say there is no law for any given type of organic behavior, because we have not yet discovered it. There is a multiplicity of these laws and their ramifications, of which, in all probability, we have no knowledge whatever. But there is one of these biologic laws of which we have the fundamental facts; that is, the law of heredity. And having the facts of this most fundamental and most potent of all biologic laws, we continue in actual practice completely to ignore it.

What is heredity? If you consult the dictionary you will get some such definition as this: "Heredity, the law according to which plants and animals inherit and transmit from generation to generation certain characteristics or tendencies." But if we give it its full biologic definition, we must say: Heredity is the force which makes and holds together the genus and the species. It determines that birds shall have wings and a special chest capacity for flight; that they shall have a bill, wide vision on all sides, etc., and for the specific bird it determines a certain plumage and a certain song, as of the robin, or the blue bird, or the thrush.

It determines that the frog shall have a special breathing apparatus to operate on land or in the water; and for the specific frog, as the bullfrog, that he shall have a certain coat color and pattern, a given size, a given call.

It goes even deeper than this, and determines that the human embryo, beginning with a single cell, like any unicellular animal or plant, divides in the same way, and in its complex cell division and differentiation recapitulates in hurried fashion the history of organic evolution.

Why? Let me here state what I conceive to be the biologic law of heredity, the law which underlies all life: *That which goes into the germ plasm, must come out in the offspring.* We must

conceive of this simple law as being as iron-clad and as immutable as a law of physics: action and reaction are equal and in opposite directions; or a law of chemistry: hydrochloric acid on zinc will produce zinc chloride. If you pour acid on metal you will get a given reaction whether you want it or not. What is put into the germ plasm will come out in the offspring, whether we want it or not—a law of nature, immutable, deaf to entreaty.

Now the unique feature of natural law is that we cannot break it. We can study it, learn to understand it and work with it, or we can continue to ignore it and combat it and be broken by it, but we cannot break it or change it; only so does the organic world hold together.

It is a general law, this law of heredity; not one law for a mouse and another for a man, another for a guinea-pig and another for a geranium, but one common law of heredity. What goes into the germ plasm comes out in the offspring, whether it is the seed of a geranium, the ovum of a guinea-pig, or of man.

And in the progress of evolution we see the constant and unbroken control of heredity, so that man, the latest product (to date) of evolution, starts with a single cell, recapitulates in his embryonal growth the history of organic evolution, and in his turn sets off the single cell (the germ plasm) made of the stuff he received from his ancestry and puts into it the identical material. This single cell (the germ plasm) in its turn again divides and in its embryonal development briefly recapitulates organic history, and in time becomes the finished example of the species; made of the material received from his ancestry—in his general build, in his length of leg, in his shape of nose, in his hair color, in the kind of kidney he has, and the kind of liver he has, and the kind of lungs and heart he has; in the kind of epithelium he has, and the kind of connective tissue, and the kind of endothelium. He starts with a vague nose shape, but it will grow into the nose shape of his ancestors; he starts with tiny legs, but they will grow to the inherited length. He inherits a liver which will in time react like the livers of his ancestors to the same causes. He inherits a type of epithelium and of connective tissue which will in time react like the epithelium and the connective tissue of his ancestors to the same causes.

So much for the general, the immutable law of heredity: What goes into the germ plasm comes out in the offspring.

How does heredity work?   As long ago as 1865 Mendel worked out with green peas the best study of the method of heredity that we have ever had.   Later, and following him, Cuenot and others worked it out with mice, and it worked out with mice exactly as it worked with peas.   Now it is vastly farther in the scheme of evolution from peas to mice than it is from mice to man.   Mice are mammals like man, their structure is like man's —a head, a trunk, four limbs.   Their organs are like man's arranged in the same relation to each other, made out of the same types of materials, functioning in the same way for the maintenance of the organism.   If we cut a mouse's arm it bleeds like man's, and then regeneration sets in as it does in man, the edges draw together, the epithelium proliferates, scar tissue is formed, which eventually either in part or wholly is absorbed—a process identical with that of man's tissues, functioning like those of a man, just as the geranium stock does if you cut it.   Why?   The law of heredity, transmitting a type of protoplasmic behavior down the full line of evolution; similar tissues functioning in the same way because they were derived from a common ancestry. If we do not accept this, we must discard the theory of evolution, for this is the heart of the theory of evolution.

Now the method of heredity as worked out by Mendel, and repeated with mice by Cuenot is this: when a pure-bred grey house mouse is crossed with a pure-bred albino, the first hybrid generation will all be grey.   That is, the tendency to pigmentation is dominant over the tendency to the lack of pigmentation. Now if we mate two of these first generation hybrid greys (heterozygotes) we shall get in the second hybrid generation some pure-breeding greys (dominants), some heterozygous greys, and some albinos (recessives), in the proportion of one to two to one. These dominant greys, if bred together or hybridized with other dominant greys similarly derived, will breed true.   The recessive albinos, whether inbred or hybridized with other pure-breeding albinos, will breed true.   The heterozygous greys, whether inbred or hybridized with other heterozygous greys simi-

larly derived, will again yield the three types, dominant greys, heterozygous greys, and recessive albinos, in the proportion of one to two to one (see chart 2, p. 118).

Again, if we cross a pure-bred albino with a heterozygous grey we shall get, in the first hybrid generation, recessive albinos and heterozygous greys in the proportion of one to one. These albinos will breed true whether inbred or hybridized with other pure-bred albinos. The heterozygous greys, in this case also, whether inbred or hybridized with other similarly derived heterozygous greys, will give the same three types: dominant greys, heterozygous greys, and recessive albinos.

Again, if we cross a dominant grey with a heterozygous grey, the first hybrid generation will give dominant greys and heterozygous greys in the proportion of one to one. The dominant greys will breed true, and the heterozygous greys will again give the same three types.

Why do these characters behave in this way? For that they do is certain. What is the secret of the method of heredity? If we mate two pure-bred albinos the offspring will all be albino; i.e., there will be a complete lack of the pigment-making mechanism. Pigment is an absent character in these individuals. It did not go into their germ plasm and they cannot transmit it to their offspring. If only albino mice are allowed to breed, the pigment-making mechanism will be lost for mice, and cannot be recovered, and there will thereafter be only albino mice. Albinism is a recessive. Pure-bred albinos cannot transmit the dominant.

Now if we mate the same pure-bred albino into whose germ plasm no pigment-making mechanism went, with a grey house mouse into whose germ plasm the tendency to pigment-making mechanism did go, we shall get in the first hybrid generation heterozygous greys, i.e., pigment-making is dominant over the lack of pigment-making, therefore the mice are grey. But into their germ plasm went from one side the absence of the pigment-making mechanism (as a unit character) and from the other side the presence of the pigment-making mechanism (another unit character); so since both these unit characters went into their

germ plasm, both of these characters will come out somewhere in the offspring—the law of heredity.

All efficient study of heredity is the study of the behavior of unit characters. Only we must be sure that we have analyzed our characteristics into unit characters, which segregate out and are transmitted as such, like albinism and pigmentation, spotting and self-color, etc. And conversely, when in the study of heredity we have found a character which does segregate out and is transmitted as such, we have found a unit character, which will behave in accordance with the immutable law of heredity for the unit character. A unit character is then to heredity what an electron is to chemistry; incapable of analysis, it segregates out and is transmitted as such.

Now when we are dealing with a complex organism like a man or a mouse, there is a multiplicity of these unit characters which have gone into his germ plasm from his ancestors, and which will get into all possible combinations. For example, a tendency to a heavy and a tall skeleton; a tendency to a particular length in the limbs; a tendency to blackness of hair with a tendency to curliness of hair; a tendency to a straight nose with a tendency to a large nose; a tendency to a certain kind of liver, which will tend to a certain type of epithelium, with a tendency to a certain type of behavior, etc.—all unit characters.

Every organism, then, is a synthesis of unit characters which cannot be correctly manipulated, or interpreted in experimental work with accurate results, until it has been analyzed into its component unit characters. Until the truth of this fact has come home to the experimental biologist, pathologist, bacteriologist, physiologist, and student of therapy, our results are certain to be invalidated by artifacts. This applies in all experimentally produced cancer; whether by grafts, or by artificial chronic irritations like painting with coal-tar products and feeding with nematode and tapeworm larvae, just as it applies everywhere else in experimental research. First by analyzing our stocks into unit characters, we must learn what portion of the result is produced by nature without reference to the experiment, and what is the experimental residuum.

With these things in mind, I undertook the study of the
heredity behavior of cancer, to find out the nature of cancer,
partly in order to learn how to get rid of a hideous disease, and
partly for the light it must throw on general biologic problems
of all tissue behavior. I proceeded to study the inheritability
of cancer in exactly this classic way, that is by making a biologic
analysis of stock, without which no stock is of any value for
conclusions concerning heredity, or practically anything else.

How do we make a biologic analysis of stock? Breed it out,
so as to find out what unit characters went into its germ plasm.
It then becomes a stock made up of analyzed individuals, whose
hereditary potentialities are known and whose effect in any
cross can be predicted.

On the other hand, if you buy animals in the market and pro-
ceed to use them for even a simple experiment, they are worthless
until they are analyzed, because some of them may be pure-bred
and some of them inevitably will be heterozygous, and they will
not behave alike in any given experiment, since they have not
the same unit characters. You have no biologic control in the
experiment until each animal to be used is analyzed.

I proceeded, then, to study the inheritability of cancer in this
way, making a biologic analysis of the stock by hybridization
and inbreeding, and I found that *equally by the method of hybridi-
zation and by inbreeding,* if you mate two mice with carcinoma of
the lung (primary or secondary) you can extract from them a
strain of 100 per cent lung tumor mice; or by mating two mam-
mary gland carcinoma mice, a strain of 100 per cent mammary
gland carcinoma can be extracted from them. That is, both in
hybridization and in inbreeding, cancer behaves like a unit
character; in other words, it segregates out and is transmitted as
such.

I tried, also, further tests of hybridization between cancer
mice and absolutely non-cancer mice. By a cancer mouse we
mean a mouse whose ancestry had cancer, into whose germ plasm
the tendency to cancer entered, and who himself has cancer.
By a non-cancer mouse we mean a mouse which came from
wholly non-cancerous parentage, into whose germ plasm there

went the tendency to the absence of cancer, and who cannot transmit the cancer tendency to his offspring. They are analyzed individuals, both of them, whose heredity behavior can be predicted.
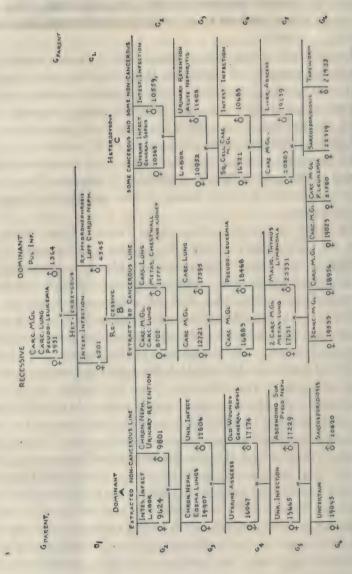
If I mate such a non-cancer mouse with a cancer mouse, into the common progeny of these two there go, first, a tendency to cancer, and second, a tendency to the absence of cancer; and the first hybrid generation can, and infallibly does, transmit some of both tendencies. But cancer is recessive to non-cancer, and so *the first hybrid generation shows none of it, and in all my experience never has shown it*, but the tendency to cancer segregates out, and in the second hybrid generation it appears again, *in the same organs and in the same tissues of those organs which show the ancestral tumors*.

Note chart 1, showing three lines derived from strain 84, branch II. This chart is perfectly typical. The parent female 3931 died of carcinoma of the mammary gland, carcinoma of the lung, and pseudoleukemia. She was *hybridized* with absolutely non-cancer male 1364 who died of pulmonary infection. In accordance with the Mendelian expectation from such a cross, no cancer appears in the first hybrid generation (cancer is recessive to non-cancer). For the parents of this branch of the family female 6201 and male 4345, both heterozygotes, were selected.

Note how there was extracted from this hybrid cross three lines of mice: (A) the dominant, which neither in direct descent nor in any accessory fraternities, ever showed one case of neoplasm, malignant or benign; (B) the recessive line, 100 per cent malignant disease, and (C) the heterozygous line showing both cancerous and non-cancerous individuals. Note how the same types and locations of neoplasms which were bred into the strain with parent female 3931, segregate out and are transmitted as such wherever tumor occurs, both in the recessive, 100 per cent cancer line B, and in the heterozygous line C, namely carcinoma of the mammary gland, carcinoma of the lung, pseudoleukemia, and its closely related tumor type, thymus lymphoma.

It is interesting to note that in the animals of this stock, chronic leukemia and pseudoleukemia (alymphatic leukemia, not

STRAIN 84 - BR.II

RECESSIVE
Carc. M.Gl.
Carc. Lung
Pseudo-Leukemia
♀ 3931

DOMINANT
Pul. Inf.
♂ 1364

G parent

Het.- Heterozygous

Re-cessive
B

Heterozygous
C

Intest. Infection
♀ 6201

Rt. Hydronephrosis
Left Chron. Neph.
♂ 4345

G₁

DOMINANT
A
EXTRACTED NON-CANCEROUS LINE

EXTRACT-ED CANCEROUS LINE

SOME CANCEROUS AND SOME NON-CANCEROUS

Intes. Infect
Labor
♀ 9624

Chron. Neph.
Urinary Retention
♂ 9801

Carc. M.Gl.
Carc. Lung
♀ 8702

Carc. Lung
Metas. Chestwall
and Kidney
♂ 11777

Uterine Infect.
General Sepsis
♀ 10945

Intest. Infection
♂ 10559

G₂

Chron. Neph.
Edema Lungs
♀ 14907

Unk. Infect
♂ 12806

Carc. M.Gl.
♀ 12121

Carc. Lung
♂ 17395

Labor
♀ 10952

Urinary Retention
Acute Nephritis
♂ 11403

G₃

Uterine Abscess
♀ 16047

Old Wounds
General Sepsis
♂ 17174

Carc. M.Gl.
♀ 16883

Pseudo-Leukemia
♂ 18466

Sq. Cell Carc.
M. Gl.
♀ 16521

Intest. Infection
♂ 10665

G₄

Unk. Infection
♀ 13665

Ascending Sup.
Pyelo Neph
♂ 11229

2 Carc. M.Gl.
Metas. Lung
♀ 17631

Malig. Thymus
Lymphoma
♂ 22531

Carc. M.Gl.
♀ 20803

Liver Abscess
♂ 19139

G₅

Uncertain
♀ 19045

Sarcosporidiosis
♂ 20820

5 Carc. M.Gl.
♀ 19533

Carc. M.Gl.
♀ 18956

Carc. M.Gl.
♂ 19023

Carc. M.Gl.
P. Leukemia
♀ 21580

Sarcosporidiosis
♀ 22319

Tapeworm
♂ 21433

G₆

SHOWING THE SEGREGATING OUT OF THE NON-CANCEROUS AND THE CANCER TENDENCIES, AND THEIR PERFECTLY TYPICAL MENDELIAN BEHAVIOR IN HEREDITY.
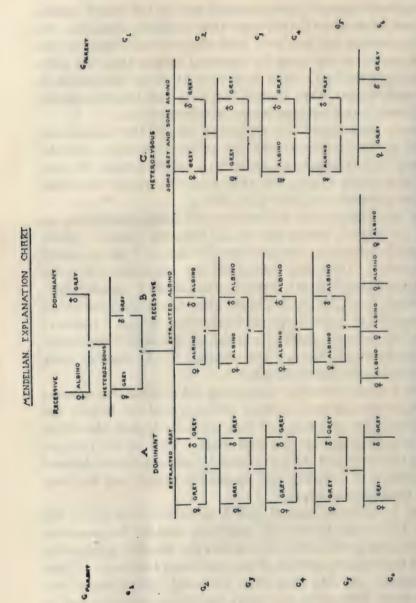
CHART I

lymphogranulomatosis) have occurred only in the cancer strains and have behaved as if they were true neoplastic diseases. As yet this material has not been fully analyzed and therefore the matter cannot at this time be discussed farther than the mere statement of the fact, which is in support of the contention of many pathologists, that chronic leukemia and pseudoleukemia are as much true neoplasms as are lymphosarcomas.

Chart 1, then, shows the segregating out and the transmission as such of the non-cancer and the cancer tendencies, also the tendency to a specificity of tissue type (which locates the neoplasm in a certain organ), and their perfectly typical Mendelian behavior in heredity as unit characters.

Note how exactly this follows the Mendelian expectation, as shown in chart 2, giving the classic behavior where a hybrid cross is made between the recessive albinism and the dominant pigmentation. Here also three lines are extracted, individual for individual parallel with those shown in chart 1: namely, first, a dominant line A, in which albinos never occurred either in the direct descent or in the accessory fraternities; second, a recessive line B, 100 per cent albinos, in which no pigmented individual ever appeared; and third, a heterozygous line C, showing some albinos and some pigmented mice.

Chart 2, then, shows the segregating out and the transmission as such, of the pigment making tendency and the lack of the pigment making tendency, and their perfectly typical Mendelian behavior in heredity as unit characters. That is, the pigment making tendency and the non pigment-making tendency behave in the matter of heredity just as did the cancer and the non-cancer tendencies in chart 1.

Chart 3 continues part of line A extracted from strain 84, branch II, *through the fifteenth generation without the occurrence of a neoplasm of any sort.* This shows the absolute segregating out and transmission as such of the non-cancer tendency. When a non-cancer line has once been extracted, spontaneous neoplasms never throughout my entire experience have occurred in such a strain again, unless cancer has again been hybridized in from an outside source. Never in line A nor in any of the accessory fraternities has a neoplasm of any sort whatever occurred.

MENDELIAN EXPLANATION CHART

RECESSIVE — DOMINANT

♀ ALBINO — ♂ GREY

HETEROZYGOUS

♀ GREY — ♂ GREY

A
DOMINANT
EXTRACTED GREY

B
RECESSIVE
EXTRACTED ALBINO

C
HETEROZYGOUS
SOME GREY AND SOME ALBINO

SHOWING THE SEGREGATING OUT OF THE PIGMENTATION AND THE NON-PIGMENTATION TENDENCIES AND THEIR PERFECTLY TYPICAL MENDELIAN BEHAVIOUR IN HEREDITY

CHART 2

STRAIN 84 – BR.A

CONTINUED THROUGH THE 15 TH GENERATION

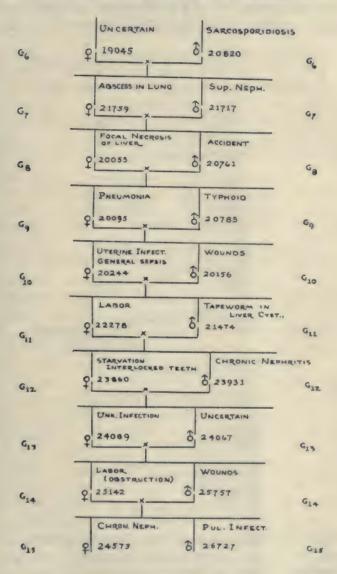|		
G_6	♀ 19045 UNCERTAIN	♂ 20820 SARCOSPORIDIOSIS	G_6
G_7	♀ 21759 ABSCESS IN LUNG	♂ 21717 SUP. NEPH.	G_7
G_8	♀ 20055 FOCAL NECROSIS OF LIVER	♂ 20761 ACCIDENT	G_8
G_9	♀ 20095 PNEUMONIA	♂ 20785 TYPHOID	G_9
G_{10}	♀ 20244 UTERINE INFECT. GENERAL SEPSIS	♂ 20156 WOUNDS	G_{10}
G_{11}	♀ 22278 LABOR	♂ 21474 TAPEWORM IN LIVER CYST.	G_{11}
G_{12}	♀ 23860 STARVATION INTERLOCKED TEETH	♂ 23931 CHRONIC NEPHRITIS	G_{12}
G_{13}	♀ 24089 UNK. INFECTION	♂ 24067 UNCERTAIN	G_{13}
G_{14}	♀ 25142 LABOR (OBSTRUCTION)	♂ 25757 WOUNDS	G_{14}
G_{15}	♀ 24573 CHRON. NEPH.	♂ 26727 PUL. INFECT.	G_{15}

CHART 3

Chart 4 continues strain 84, branch II, line B, through the ninth generation. This is the 100 per cent cancer line extracted from this same cross. Note how the types and locations of the neoplasms occurring in this line of the strain are the same as

STRAIN 84 – BR.II Part of Line B

CONTINUED TO THE 9TH GENERATION

G4	CARC. M.GL. ♀ 16883	PSEUDO LEUKEMIA ♂ 18468	G4
G5	2 CARC M.GL METAS LUNGS ♀ 17631	MALIG. THYMUS LYMPHOMA ♂ 22331	G5
G6	CARC. M.GL PSEUDO LEUKEMIA ♀ 21580	ADENOMA LUNG ♂ 17389	G6
G7	CARC. M GL. ♀ 20803	SP.CELL SARCOMA MEDIASTINUM AND PERITONEUM. ♂ 20102	G7
G8	CARC M.GL. ♀ 21755	PSEUDO LEUKEMIA ♂ 25951	G8
G9	PSEUDO LEUKEMIA ♀ 21661		G9

CHART 4

those bred into it in the original cross from female 3931, namely, carcinoma of the mammary gland, carcinoma of the lung, and pseudoleukemia, with its closely related tumor type, thymus lymphoma. Note female 21580 (generation 6) with the carci-
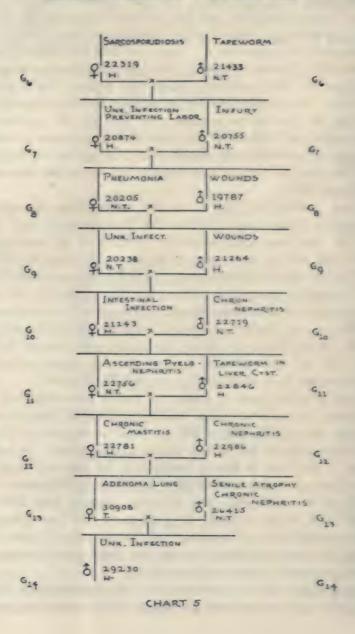
noma of the mammary gland of her grandmother and the pseudo-leukemia of her grandfather. Note also the interesting sequence here of pseudoleukemia and thymus tumors through six consecutive generations, following the selection of male 18468 as the parent male in generation 4. Note also that the original parent female 3931 (shown in chart 1) had pseudoleukemia along with carcinoma of the mammary gland and primary carcinoma of the lung. Note male 20102 in generation 6, with a spindle cell sarcoma of the entire mediastinum and of the peritoneum. This single case of sarcoma was derived from an ancestor several generations antecedent to female 3931, the parent of strain 84. Lack of space prevents the showing of this ancestral sequence in the single chart.
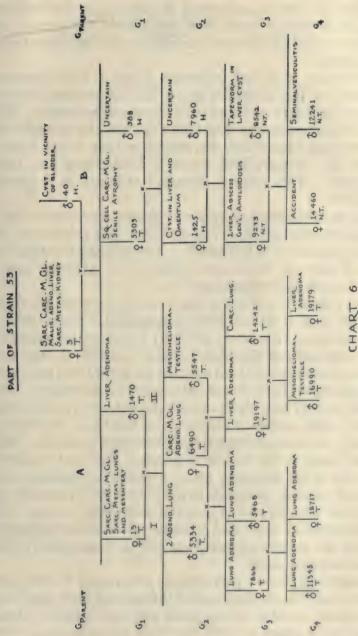
Chart 5 continues line C of this same strain through the fourteenth generation. This is the heterozygous line. Note how, by the continued selective breeding of a heterozygous individual with a non-cancerous mate (as indicated by H and $N.T.$ in the chart) all occurrence of neoplasms was held off until the thirteenth generation. Here, by the mating of two mice heterozygous to lung tumor, lung tumor occurred in the thirteenth generation, the parents concerned being female 22781 and male 22986. Lung tumor is one of the tumor types carried by this line. *By the right selective breeding in any heterozygous line, neoplasms can be made to occur or can be held off at will.*

Chart 6 shows part of strain 53, line A branching into two extracted 100 per cent cancer families, and line B, from the same parents, developing into a 100 per cent non-cancerous family. The heterozygous line is not shown in this chart, for lack of space. Note how, in line A family 1, a 100 per cent lung adenoma family is being extracted by the selection as parents of the family of two mice with lung adenoma, namely male 5334 and female 6490; while in family II of the same line, liver adenomas and mesotheliomas of the testicle are the prevailing tumors, there being four liver adenomas in the ten individuals forming the direct descent of these five generations.

Note that in line B after the first hybrid generation (female 5303 with a squamous cell carcinoma of the mammary gland)

STRAIN 84 – BR.C
CONTINUED THROUGH THE 14TH GENERATION

	SARCOSPORIDIOSIS	TAPEWORM	
G6	♀ 22319 H.	♂ 21433 N.T	G6
G7	UNK. INFECTION PREVENTING LABOR	INJURY	
	♀ 20874 H x	♂ 20755 N.T.	G7
G8	PNEUMONIA	WOUNDS	
	♀ 20205 N.T. x	♂ 19787 H.	G8
G9	UNK. INFECT.	WOUNDS	
	♀ 20238 N.T x	♂ 21264 H.	G9
G10	INTESTINAL INFECTION	CHRON NEPHRITIS	
	♀ 21243 H. x	♂ 22719 N.T.	G10
G11	ASCENDING PYELO- NEPHRITIS	TAPEWORM IN LIVER CYST.	
	♀ 22756 N.T. x	♂ 22846 H	G11
G12	CHRONIC MASTITIS	CHRONIC NEPHRITIS	
	♀ 22781 H x	♂ 22986 H	G12
G13	ADENOMA LUNG	SENILE ATROPHY CHRONIC NEPHRITIS	
	♀ 30908 T. x	♂ 26415 N.T	G13
G14	UNK. INFECTION		
	♂ 29230 H-		G14

CHART 5

PART OF STRAIN 53

A
B

CHART 6

G PARENT

G₁

G₂

G₃

G₄

Left block (A):

- G_{PARENT} — SARC. CARC. M. GL. SARC. METAS. LUNGS AND MESENTERY ♀ 15 T. — I
- LIVER ADENOMA ♂ 1470 T. — II
- CARC. M. GL. ADENO. LUNG ♀ 6490 T.
- 2 ADENO. LUNG ♂ 5334 T.
- MESOTHELIOMA TESTICLE ♂ 5547 T.
- LIVER ADENOMA ♀ 19197
- LUNG ADENOMA ♂ 5468 T.
- LUNG ADENOMA ♀ 7866 T.
- CARC. LUNG. 14242 T.
- LIVER ADENOMA ♂ 19179 T.
- MESOTHELIOMA TESTICLE ♂ 16990 T.
- LUNG ADENOMA ♀ 18717 T.
- LUNG ADENOMA ♂ 11345 T.

Right block (B):

- G_{PARENT} — SARC. CARC. M. GL. MALIG. ADENO. LIVER SARC. METAS. KIDNEY ♀ 3 T.
- CYST IN VICINITY OF BLADDER ♂ 40 H.
- SQ. CELL CARC. M. GL. SENILE ATROPHY ♀ 5303 T.
- UNCERTAIN ♂ 388 H.
- CYST IN LIVER AND OMENTUM ♀ 1425 H.
- UNCERTAIN ♂ 7960 H.
- LIVER ABSCESS GEN'L AMYLOIDOSIS ♀ 9233 N.T.
- TAPEWORM IN LIVER CYST ♂ 9542 N.T.
- ACCIDENT ♀ 14460 N.T.
- SEMINAL VESICULITIS ♂ 12241 N.T.

there was no further appearance of tumor. By the continued selection of non-tumorous individuals after the second filial generation, all neoplasms were completely ruled out of this branch of the strain.

Note female 13 of filial generation 1, line A, with a sarcoma-carcinoma of the mammary gland like her mother, female 3, and with secondary sarcomas in the lungs and mesentery. Note how, with her secondary lung tumor she was able to start a 100 per cent lung tumor line, the secondary lung tumor being as efficient as a primary lung tumor in transmitting lung tumor potentiality. This point is discussed at length in a previous report (16).

Note the prevalence of cyst and abscess formation in line B of strain 53, although in no case do these cysts or abscesses lead to tumor formation in this family. The offspring is made of the identical material of the germ plasm of its ancestry, and its tissues behave in the same way.

Chart 7 shows line B of this strain *continued through the eleventh generation without the occurrence of neoplasms.* Never, either in the direct descent nor in any accessory fraternity, did a neoplasm of any kind occur in this branch of strain 53 after the neoplastic tendency had once been bred out from the second filial generation.

Chart 8 shows part of strain 215 and some derivatives. Note the 100 per cent lung tumor strain being extracted in line A from female 5 with secondary carcinoma of the lung. Note the 50 per cent liver adenoma strain being extracted in line B. Strain 215 was made by the mating of female 3, who had a sarcoma-carcinoma of the mammary gland, a malignant adenoma of the liver, and sarcoma metastasis in the kidney, with male 360, who was proved heterozygous to lung and mediastinal tumors. Note how the different types and locations of neoplasms introduced by these two parents segregate out and are transmitted as such in the succeeding strain.

Charts 9 and 10 show part of strain 338, branch V with partial ancestry, and its offspring carried through the ninth generation. The original ancestor of this strain also was female 3, already referred to in chart 8. She had a sarcoma-carcinoma of the

STRAIN 53 – LINE B
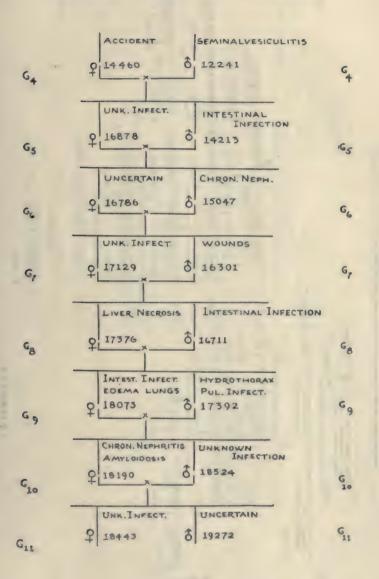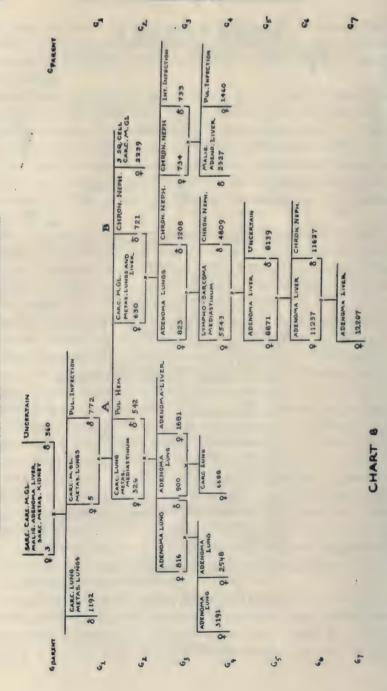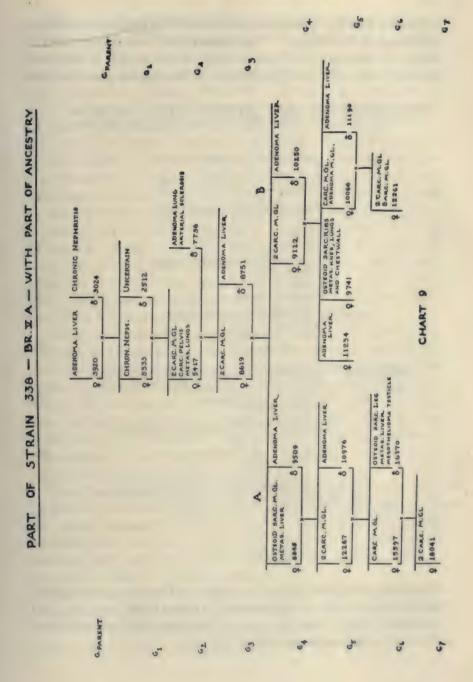
CONTINUED THROUGH 11TH GENERATION

	ACCIDENT	SEMINALVESICULITIS	
G_4	♀ 14460	♂ 12241	G_4
	UNK. INFECT.	INTESTINAL INFECTION	
G_5	♀ 16878	♂ 14213	G_5
	UNCERTAIN	CHRON. NEPH.	
G_6	♀ 16786	♂ 15047	G_6
	UNK. INFECT.	WOUNDS	
G_7	♀ 17129	♂ 16301	G_7
	LIVER NECROSIS	INTESTINAL INFECTION	
G_8	♀ 17376	♂ 16711	G_8
	INTEST. INFECT. EDEMA LUNGS	HYDROTHORAX PUL. INFECT.	
G_9	♀ 18073	♂ 17392	G_9
	CHRON. NEPHRITIS AMYLOIDOSIS	UNKNOWN INFECTION	
G_{10}	♀ 18190	♂ 18524	G_{10}
	UNK. INFECT.	UNCERTAIN	
G_{11}	♀ 18443	♂ 19272	G_{11}

CHART 7

PART OF STRAIN 215 AND SOME DERIVATIVES.

G PARENT

SARC. CARC. M.GL.
MALIG. ADENOMA LIVER.
SARC. METAS. KIDNEY
♀ 3

UNCERTAIN
♂ 360

PUL. INFECTION
♂ 772

A

CHRON. NEPH.
♂ 721

J. SQ. CELL
CARC. M.GL.
♀ 2239

B

CARC. LUNG
METAS. LUNGS
♂ 1192

CARC. M.GL.
METAS. LUNGS
♀ 5

PUL. HEM.
♂ 542

CARC. M.GL.
METAS. LUNGS AND
LIVER.
♀ 630

INT. INFECTION
♂ 733

CHRON. NEPH.
♂ 734

PUL. INFECTION
♀ 1460

MALIG.
ADENO. LIVER.
♂ 2327

CARC. LUNG
METAS.
MEDIASTINUM
♀ 326

ADENOMA-LIVER.
♂ 1681

ADENOMA LUNGS
♀ 823

CHRON. NEPH.
♂ 1208

ADENOMA LUNG
♂ 816

ADENOMA
LUNG
♂ 900

CARC. LUNG
♀ 4188

LYMPHO - SARCOMA
MEDIASTINUM
♀ 5543

CHRON. NEPH.
♂ 4809

ADENOMA
LUNG
♀ 3191

ADENOMA
LUNG
♀ 2548

ADENOMA LIVER.
♀ 6871

UNCERTAIN
♂ 6139

ADENOMA LIVER.
♀ 11237

CHRON. NEPH.
♂ 11027

ADENOMA LIVER.
♀ 12207

G PARENT

G₁

G₂

G₃

G₄

G₅

G₆

G₇

CHART 8

PART OF STRAIN 338 — BR. II A — WITH PART OF ANCESTRY

G. PARENT

G₁

G₂

G₃

G₄

G₅

G₆

G₇

A

ADENOMA LIVER
♂ 9509

OSTEOID SARC. M. GL.
METAS. LIVER
♀ 8965

ADENOMA LIVER
♂ 10976

2 CARC. M. GL.
♀ 12267

CARC. M. GL.
♀ 15597

OSTEOID SARC. LEG
METAS. LIVER
MESOTHELIOMA TESTICLE
♂ 16370

2 CARC. M. GL.
♀ 16041

ADENOMA LIVER
♀ 3920

CHRONIC NEPHRITIS
♂ 3024

CHRON. NEPH.
♀ 5333

UNCERTAIN
♂ 2512

2 CARC. M. GL.
CARC. PELVIS
METAS. LUNGS
♀ 5417

ADENOMA LUNG
ARTERIAL SCLEROSIS
♂ 7756

2 CARC. M. GL.
♀ 6619

ADENOMA LIVER
♂ 6751

B

ADENOMA LIVER
♂ 10250

2 CARC. M. GL.
♀ 9112

ADENOMA
LIVER
♀ 11254

OSTEOID SARC. RIBS
METAS. KNEE, LUNGS
AND CHESTWALL
♀ 9741

ADENOMA LIVER
♂ 11190

CARC. M. GL.
ADENOMA M. GL.
♂ 10066

2 CARC. M. GL.
SARC. M. GL.
♀ 12261

CHART 9

mammary gland, a malignant adenoma of the liver, and sarcoma metastasis in the kidney.   Note the striking outcropping of liver tumors in this strain.   Note how the different unit characters, sarcoma, carcinoma, and specificity of liver tissue type, get into

CONTINUATION   OF   STRAIN 338 — BR. $\underline{\text{V}}$ A.

G_3

2 CARC M.GL ♀ 8619 × ‎ ADENOMA LIVER ♂ 8751

G_3

G_4

2 CARC. M.GL. ♀ 9112 ADENOMA LIVER ♂ 10250

G_4

G_5

CARC. M.GL. ADENOMA M.GL ♀ 10066 ADENOMA LIVER ♂ 11190

G_5

G_6

2 CARC. M.GL. SARC. M.GL ♀ 12261 CARC. LUNG ♂ 21291

G_6

G_7

ADENOMA LUNG ♀ 17681

P. LEUKEMIA ♂ 19913

3 CARC. M.GL — PERITONEAL SARC. METAS. LIVER , PANCREAS AND UTERINE WALL ♀ 22263

G_7

G_8

CARC. M.GL ♀ 24274

SARCOMA HEAD AND NECK ♂ 26259

G_8

G_9

7 CARC. M.GL.- LIVER ADENOMA LUNG ADENOMA - UTERINE FIBROID DIFFUSE ENDOTH. HYPERPLASIA OF GLANDS AND SPLEEN ♀ 30469

2 CARC. M.GL. LIVER NEARLY REPLACED BY METAS. ♀ 30501

G_9

CHART 10

all possible combinations in the strain, so that even in the small number of individuals represented in these two charts, we find carcinoma of the liver, sarcoma of the liver, both primary and secondary, and adenoma of the liver.

That is, the carcinoma tendency segregates out and is transmitted as such.   The sarcoma tendency segregates out and is transmitted as such.   The adenoma tendency segregates out and is transmitted as such.   A specificity of liver tissue which will insure its yielding to neoplasms, segregates out and is transmitted as such.   These are all unit characters, and they get into all possible combinations even in this one small family alone.

I have from necessity selected only a few strains for the charts in this report.   They are perfectly typical, however, and are representative of the hundreds of other strains, tumorous and non-tumorous, inbred and hybridized, which have been produced by selective breeding alone, and which have been analyzed in this laboratory for the past fifteen years.   I have therefore been able to express the matter in little more than outline, and there are many and diverse ramifications of the work which, for the sake of unity, I cannot enter into at all at this time.   But certain facts have consistently obtained in this work for twelve years, and these I wish to emphasize with their application.

Cancer and non-cancer have behaved consistently just as true albinism and pigmentation do in heredity.   That is, just as true albinism is the total absence of the pigment-making mechanism present in the pigmented mouse, so *spontaneous cancer consistently behaves like the absence of a mechanism fitted to control proliferation and differentiation in regenerative processes.*   At any rate, whether or not it is exactly this, it seems to be the absence of some controlling mechanism, and an animal either has it or has not, whether he is a mouse or a man.

Whenever spontaneous cancer comes out in a strain, it is because it has been bred in, in some degree; and however remote the cancer ancestry, we shall find it if we analyze far enough. *Moreover, we shall find not only the neoplastic ancestor, but the ancestors that carried the same types and the same locations of neoplasms shown in the later generations.*

There is no appearance of spontaneous cancer in any non-tumor strain.   There are all percentages of cancer, some even as low as 0.1 in varying cancer strains.   That is, cancer and non-cancer behave respectively like the absence and presence of a

particular controlling mechanism and an individual, whether it is a mouse or a man, either has it or lacks it.

The unit characters concerned in the heredity of spontaneous tumors, whether we are dealing with mice, with rats, or with man, are these: First, specificity of tissue type from organ to organ, which determines that the liver, or the kidney, or the uterus, or the mammary gland tissue, and so forth, shall be like the tissue of its ancestral organ (from which it was derived) and shall react in the same way to the same cause. For example, liver tumor begets liver tumor, etc. Second, a specificity of epithelium from ancestor to offspring which shall cause it to proliferate without differentiation and without control under a given provocation, i.e., carcinoma begets carcinoma. Third, a specificity of connective tissue from ancestor to offspring, which shall cause it to proliferate without differentiation and without control under a given provocation, i.e., sarcoma begets sarcoma.

Like all other unit characters, the unit characters here enumerated may get into all possible combinations; and we therefore, when dealing with fundamentally and completely analyzed stocks, have such a result as I have shown from female 3. This female 3, with a sarcoma-carcinoma of the mammary gland, a malignant adenoma of the liver, and sarcoma metastasis in the kidney, is able to transmit to her posterity (and has so transmitted) all possible combinations of these unit characters, namely: carcinoma of the mammary gland, sarcoma of the mammary gland, sarcoma-carcinoma of the mammary gland, adenoma of the mammary gland; carcinoma of the liver, sarcoma of the liver, adenoma of the liver; carcinoma of the kidney, sarcoma of the kidney, adenoma of the kidney.

There are only two possible methods of studying the inheritability of any character whatever, cancer or anything else. These are: First, the long, painstaking, difficult analysis of stock in the laboratory, so that we obtain analyzed individuals whose hereditary potentialities are known quantities and can be manipulated as such. This is the method which has been pursued for fifteen years in this laboratory. And second, the so-called statistical method which has been in vogue in the study of the inheritability of cancer in man.

All human statistics of this nature are based upon two things both of which may be in error. Namely, the memory of the patient, and the diagnoses concerning his ancestry. Rarely, back of one generation, are the facts remembered or the diagnoses based upon autopsy. We have then no certain scientific material whatever to deal with in these statistics.

But where these statistics are right, as they frequently even by chance must be, a biologic reading of them would show that they also demonstrate the inheritability of cancer in man.

Note chart 11 showing part of Strain 164 with partial ancestry in the maternal side. *Female 1236 who died of a thrombosed auricle,[2] was the parent female* of strain 164. Her mother was tumorous (indicated by *T* in the chart) and died of a highly malignant carcinoma of the mammary gland with metastases in the lungs. Her father, male 242, died of uncertain causes. He did not himself have tumor but was proved heterozygous to tumor (indicated by *H* in the chart); that is, he inherited it from cancerous ancestry and transmitted it to his offspring although not himself having tumor.

The male parent of strain 164 was a pure-bred house mouse, and died of uncertain causes. He was a member of strain 358, in my hands many years without the occurrence of tumor of any sort, malignant or benign, in any of its fraternities. He himself was an analyzed, proved non-tumorous mouse (indicated by *N.T.* in the chart).

In the first hybrid generation from this cross (namely, between a heterozygote and a non-tumorous mouse) no tumor of any sort has ever occurred. Cancer is recessive to non-cancer. In this chart the only members of the first hybrid generation of strain 164 that are shown are two pairs, namely, female 4224 and male 6480, the parents of branch III of the strain; and female 4921 and male 5652, parents of branch I of the strain. Female 4224 and male 6480 of branch III were both proved heterozygous to cancer, while female 4921 and male 5652, parents of Branch I, were both proved non-tumorous mice.

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[2] This is a condition that has occurred with some frequency in this laboratory.

Note that the progeny in branch I (with both parents non-tumorous) were all absolutely non-tumorous. Nowhere, either in the direct descent nor in any of the accessory fraternities in this branch of the strain, has there ever been a single occurrence of tumor of any sort, malignant or benign. This is the classic

PART OF STRAIN 164 WITH PARTIAL ANCESTRY
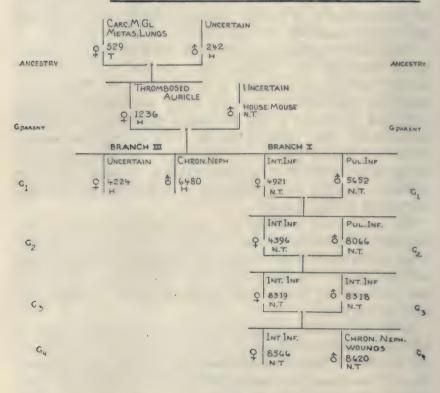
CHART 11

Mendelian behavior to be expected from the mating of two individuals carrying the dominant, that is, the non-cancer tendency, like female 4921 and male 5652. Branch I, then, strain 164, is an absolutely non-tumorous family, every member of which has been analyzed and has proved to be non-tumorous both in inbreeding and in hybridization, although this branch was

derived from cancer ancestry on the maternal side.   This shows conclusively that the non-cancer tendency also segregates out and is transmitted as such, and that by the right selective mating the cancer tendency can be ruled out absolutely from a family, beginning with the second hybrid generation.

Chart 12 shows the origin of branch III of this same strain 164, and the parentage originating the three lines A, B, and C, in which this branch has been bred out.   The succeeding charts will show the continuation of these three lines.

The parents of line A were tumorous male 3672 who died of a lymphosarcoma of the thymus, and heterozygous female 8419 who died of peritonitis.   Their two offspring selected to carry on this line were both heterozygous to tumor, namely, female 10597 who died of tapeworm, and male 8521 who died of intestinal infection.

The parents of line B were non-tumorous female 7126 and heterozygous male 6728.   Their offspring selected to carry on line B were heterozygous female 8146 and non-tumorous male 8150, both dying of intestinal infection.

The parents of line C were heterozygous female 7913 and tumorous male 8276 who died of sarcoma of the mammary gland. Their offspring selected to carry on line C were tumorous female 10537 with a carcinoma of the mammary gland, and non-tumorous male 8247 who died of chronic nephritis.   This chart, then, shows how lines A, B, and C of branch III, strain 164, originated.

Chart 13 shows line A of branch III, strain 164, continued through eight generations.   Note now, how by the right selective matings of heterozygous and non-tumorous mice, the occurrence of malignant disease is held off until the sixth generation. If, now, female 12876 with a lymphosarcoma of the mesentery, left kidney, and right ovary, had had her statistics taken in the hospital without error even for three generations, no statistics of tumor would have appeared; nevertheless the inheritance of her tumor type is direct from her grandfather four generations back. By the mating of two heterozygous individuals from this tumor mother, namely female 11551 and male 11440, again in the next

PART OF STRAIN 164-BRANCH III
SHOWING HOW LINES A, B AND C ORIGINATED

ANCESTRY

G PARENT

G_1

LINE C

UNCERTAIN | ♂ 7125 N.T. | G_2

SARC. M. GL. | ♂ 8276 T | G_3

INT. INF. | ♀ 7913 H

CHRON. NEPH. | ♂ 8247 N.T.

CARC. M. GL. | ♀ 10537 T.

UNK. INF. | ♂ 7101 H

LINE B

CHRON. NEPH. | ♂ 6728 H

INT. INF. | ♀ 7126 N.T.

INT. INF. | ♂ 8150 N.T.

INT. INF. | ♀ 8146 H

CHRON. NEPH. | ♂ 6480 H

BRANCH III

ANCESTRY

CARC. M. GL. METAS. LUNGS | ♀ 529 T

UNCERTAIN | ♂ 242 H

THROMBOSED AURICLE | ♀ 1236 H

UNCERTAIN | ♂ House Mouse N.T.

G PARENT

UNCERTAIN | ♀ 4224 H

PERITONITIS | ♀ 8419 H

LINE A

LYMPHO SARCOMA THYMUS | ♂ 3672 T | G_1

INT. INF. | ♂ 8521 H | G_2

TAPEWORM | ♀ 10597 H | G_3

CHART 12

PART OF STRAIN 164 – BR. III
Line A Continued Through 8 Generations

LYMPHO-SARCOMA THYMUS	PERITONITIS	
♂ 3672 T	♀ 8419 H	
TAPEWORM	INT. INF.	
♀ 10597 H	♂ 8521 H	
CHRON. NEPHRITIS	APPENDICITIS	
♀ 10805 H	♂ 8597 N.T.	
LABOR INT. INF.	CHRON. NEPH.	
♀ 11195 H	♂ 11322 H	
LYMPHO-SARC. MESENTERY LEFT KIDNEY AND RIGHT OVARY	WOUNDS	
♀ 12876 T	♂ 11380 N.T.	
INT. INF.	INT. INF.	
♀ 11551 H	♂ 11440 H	
LYMPHOMA THYMUS		
♀ 18365 T		

G₂ ... G₂
G₃ ... G₃
G₄ ... G₄
G₅ ... G₅
G₆ ... G₆
G₇ ... G₇
G₈ ... G₈

CHART 13

generation a malignant thymus lymphoma occurs in female 18365. At the same time, all other types and locations of tumors are ruled out from this strain by the right selective matings.

Chart 14 shows line B of branch III, strain 164, continued through nine generations. Note how by continued matings of heterozygous and non-tumorous individuals, all occurrence of malignant disease has been held off until the eighth generation. If female 21189 of the eighth generation, who died of an osteosarcoma of the leg and spine, had had her hospital statistics taken even seven generations without error, there would have been no appearance of tumor. Nevertheless, by tracing far enough back, we find the ancestor with malignant disease. This chart shows with what certainty the tendency to neoplasms can be manipulated and controlled. Through generation after generation it was carried along by heterozygous individuals, certain to appear when the right matings were made.

Chart 15 shows line C of this branch of strain 164 continued through seven generations. Here by mating a tumorous female 10537 with a proved non-tumorous male 8247, no tumor occurred in the next generation. But by the selection of two heterozygotes of generation 4, carcinoma appeared in the succeeding generation (generation 5) in female 12779 with a squamous cell carcinoma of the neck and pseudoleukemia. Again, by the mating of this female with a heterozygous male 16667, note how both pseudoleukemia occurred in the next generation, male 14631, and also squamous cell carcinoma of the neck, which at death had extended anteriorly and obliterated the face (male 14583). Female 18413, daughter of male 14583, showed the carcinoma of the mammary gland of her grandmother four generations back and the sarcoma of her grandfather five generations back. When a given type or location of neoplasm is bred in, it can be manipulated with absolute accuracy by the type of selective breeding used. It can be made to hold off for any number of generations desired or to appear in the next generation, as in the case of male 14583 with a squamous cell carcinoma of the neck in generation 6, following female 12779 with a squamous cell carcinoma of the neck in generation 5; or male 14631

PART OF STRAIN 164 BR III
LINE B CONTINUED THROUGH 9 GENERATIONS

G_2 ♀ | 7126 INT. INF. N.T.   ♂ | 6728 CHRON. NEPH. H G_2

G_3 ♀ | 8146 INT. INF. H   ♂ | 8150 INT. INF. N.T. G_3

G_4 ♀ | 8917 CHRON. NEPH. H   ♂ | 8362 WOUNDS N.T G_4

G_5 ♀ | 10479 ACCIDENT H   ♂ | 8790 ACCIDENT N.T. G_5

G_6 ♀ | 11119 SUP. NEPH. N.T.   ♂ | 10035 INT. INF. H G_6

G_7 ♀ | 12689 LABOR H   ♂ | 12853 CHRON. NEPH. H G_7

G_8 ♀ | 21189 OSTEO. SARC. LEG AND SPINE T   ♂ | 20770 WOUNDS N.T. G_8

G_9 ♀ | 16660 PUL. INF. H   ♂ | 22595 ABSCESS LUNG H G_9
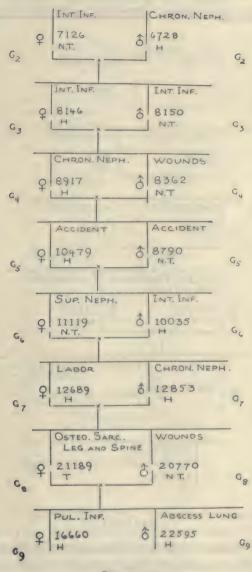
CHART 14

with pseudoleukemia in generation 6 following female 12779
with pseudoleukemia in generation 5.

PART OF STRAIN 164 – BR. III
LINE C CONTINUED THROUGH 7 GENERATIONS
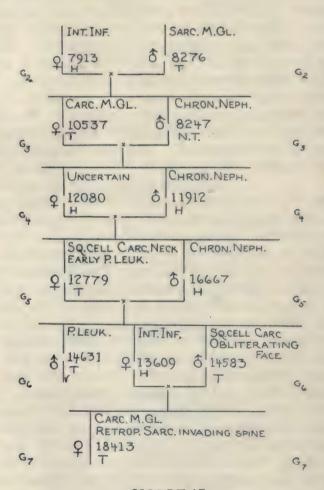
CHART 15

If human cancer statistics when correctly taken were biolog-
ically read, they would show as certainly as do mouse statistics
the inheritability of cancer.　They would show that the human

heterozygote carries and transmits neoplastic tendencies exactly as do mouse heterozygotes, although they themselves do not develop the disease. This follows exactly the classic Mendelian pattern from the mating of pigment-bearing with non-pigment-bearing mice.

PART OF STRAIN 392
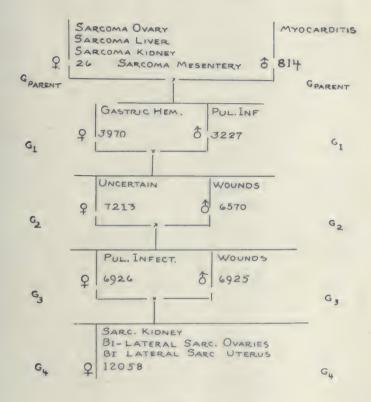
CHART 16

Chart 16 shows part of strain 392. The parent female 26 had a sarcoma of the ovary, sarcoma of the liver, sarcoma of the kidney and perirenal tissues, and a sarcoma of the mesentery. She was mated with male 814 who died of myocarditis. By the right selective mating, the occurrence of sarcoma (and all other types

of neoplasms) was held off through the three succeeding genera-
tions. In the third hybrid generation, by the selection of two
individuals heterozygous to sarcoma, sarcoma appeared in the
immediate offspring (generation 4), female 12058 repeating the
sarcoma of the kidney and of the ovaries of her grandmother
four generations back, adding also bilateral sarcoma of the
uterus. No present day hospital statistics could have shown the
correct causes of death through four generations. If, therefore,
this had been a human case, there would have been no record of
tumorous ancestry—yet there is here the most evident and per-
fect persistence and final emergence of the exact type and loca-
tions of neoplasms, through the right selective breeding to bring
it out.

Again note chart 17, showing strain 465 with partial ancestry.
Let me go through this chart somewhat in detail, in order that
we may see the perfect evidence it affords of *first, the segregating
out of tumor types and their consequent inheritability, and second,
the segregating out and consequent inheritability of a specificity of
organ tissue type, transmitted through generation after generation
both where inbreeding and where hybridization was employed.*

Female 3 and male 30 were the ancestors of the paternal side of
this strain. Female 3 had a sarcoma-carcinoma of the mammary
gland, a malignant adenoma of the liver, and sarcoma metastasis
in the kidney. Male 30 was proved heterozygous to tumor.
This is a case, then, of mating a tumorous individual (recessive)
with a heterozygote. In accordance with the Mendelian ex-
pection from such a cross, tumor comes out in the first hybrid
generation; namely, female 883 with an adenoma of the liver.

In the first filial generation this female 883, with an adenoma
of the liver, was *hybridized* with male 842 who died of uncertain
causes, but who was proved heterozygous to tumor. Their
son, male 1011, dying from acute nephritis, was heterozygous to
liver tumor. He was *hybridized* with female 441 (entirely
unrelated) who was also heterozygous to tumor. Their son,
male 3024, was *hybridized* with female 3920, who came of a liver
tumor ancestry and who herself had an adenoma of the liver.
Note the outcropping of liver tumor in the second hybrid genera-

PART OF STRAIN 465—WITH PARTIAL ANCESTRY

G PARENT ANCESTRY	♀ Sarc. Carc. M.Gl. Malig. Adenoma Liver Sarc. Metas. Kidney 3	♂ Chron. Neph. 30 G. PARENT ANCESTRY
G_1	♀ Adenoma Liver 883	♂ Uncertain 842 G_1
G_2	♀ Chron. Neph. 441	♂ Urinary Retention Acute Neph. 1011 G_2
G_3	♀ Adenoma Liver 3920	♂ Chron. Neph. 3024 G_3
G_4	♀ Chron. Neph 5333	♂ Uncertain 2512 G_4
G PARENT STRAIN 465	♀ Sarc. Liver 5305	♂ Chron. Neph. 5215 G. PARENT STRAIN 465
G_1	♀ Accident 5883	♂ Uncertain 6369 G_1
G_2	♀ Uncertain 9957	♂ Chron. Neph. Senile Atrophy 8888 G_2
G_3	♀ Chron. Neph. 9847	♂ Lung Abscess 8852 G_3
G_4	♀ Sarc. Liver and Common Bile Duct Lympho-Sarcoma Stomach 11252	G_4

CHART 17

tion, female 5305 with a sarcoma of the liver. Note here how there segregated out on the one hand the unit character sarcoma introduced by female 3, five generations back, and on the other hand the specificity of liver tissue type locating tumors in the liver which also was originally introduced by female 3, transmitted through and reinforced by female 883 and female 3920. Note how in this generation the unit character sarcoma got into combination with liver tissue of a type to yield to neoplasmic growth (another unit character), so that we have in female 5305 a *sarcoma* of the *liver*.

This female 5305 was *hybridized* with male 5215, who died of chronic nephritis. Through three succeeding generations, by the right selective matings, all occurrence of neoplasms was held off. But the certainty of its transmission by heredity is indisputably demonstrated, for by the use of analyzed individuals two mice heterozygous to liver tumor were selected in the third filial generation, namely female 9847 who died of chronic nephritis, and male 8852 who died of a lung abscess; and liver tumor appeared in the next generation. Not only liver tumor occurred, but the same combination of unit characters occurred, *namely the combination of sarcoma, and the neoplastic tendency in the liver.*

Let me summarize the facts demonstrated in this chart (chart 17).

1. Sarcoma segregated out and was transmitted as such.

2. Adenoma segregated out and was transmitted as such.

3. Other types of neoplasms segregated out and were not transmitted at all in this line of succession.

4. A specific type of liver tissue (viz., lacking the non-cancer mechanism) segregated out and was transmitted as such, so that five liver tumors occurred in this small family alone.

5. A specific type of tissue in all other organs (viz., possessing the non-cancer mechanism) segregated out and was transmitted as such, so that all other organs refused neoplastic growth, with the single exception of female 11252, whose liver sarcoma spread by extension into the common bile duct, and who had also a lymphosarcoma of the stomach; but it is notable that this is the family in which nearly all of the few stomach tumors in this stock have occurred.

In the light of such perfect evidence as this, it is logically absurd to question the segregating out as unit characters of the sarcoma tendency, the carcinoma tendency, adenoma tendency, and the tendency to a specific type of organ tissue determining the location of neoplasms, and their transmission as such by heredity. Moreover, when we have such analyzed human individuals and such exact data concerning their neoplasms (if such a time ever comes) *we shall find that exactly the same laws govern the transmisson and occurrence of human neoplasms, otherwise there is no such thing as biologic or organic law.*

Charts 11 to 17 inclusive show how the tendency to neoplasms of specific types and of specific organs is carried along by heterozygotes, and how by the right selective matings alone, both in inbreeding and in hybridization, neoplasms of these types and of these organs can be held off or brought out at will in the resulting strains by the use of analyzed individuals. Such neoplastic tendencies can be carried along by heterozygous individuals through any number of generations desired, just as the tendency to albinism can be carried along by heterozygotes as long as may be desired. But by right selective breeding, both the neoplastic tendency and the albinic tendency can be made to emerge at will.

The heterozygote, then, the product of hybridization in any species or any variety, in whom the recessive (cancer or albinism etc.) lies hidden but potent for transmission, may be a very puzzling factor in heredity; for he contains in his germ plasm, and therefore can transmit to his offspring, unit characters different and frequently opposite in nature. As, for example, a pair of heterozygous black mice transmitting albinism to their immediate offspring, or a pair of heterozygous non-cancer mice transmitting cancer to their immediate offspring, because potential cancer went into the germ plasm from which the heterozygotes developed. The heterozygotes in the human cancer problem have been the individuals who have blinded the readers of human statistics to the fact of the inheritability of human cancer, hiding as they do the recessive (cancer) behind the dominant appearance, which is appearance only.

There is a very widespread objection in the medical profession today to the thought that cancer is inheritable, and a very widespread and ready categorical denial of its inheritability, on the basis of these erroneous and misread human cancer statistics. As the opinion of many physicians and surgeons in this matter is based upon the statements made by the Society for the Control of Cancer, let me here quote from this year's annual leaflet put out by the Society, item no. 6: "Cancer is not inherited. It is not certain even that a tendency to the disease is inherited."

Let us subject this excerpt to the biologic test. I have reminded you that man repeats in his embryonal development the history of organic evolution. He begnis as a single cell. In this cell there is no nose, no legs, no vertebrae arranged in a perfect and beautiful spinal column, no liver, no epithelium, no cancer. What resides in this single cell is the tendency to all these things. That is the basis of all heredity. All inherited characters are inherited tendencies of the cell. There is no other form of inheritance.

I have emphasized the evolutionary basis of the law of heredity, a common law of protoplasmic behavior—What goes into the germ plasm comes out in the offspring; the fundamental necessity of similar tissues behaving in similar fashion if there is to be such a thing as species or race. The mouse tumors under study in this laboratory are spontaneous tumors, arising in the natural life of the animal without artificial interference of any sort except that of selective breeding, exactly as man's tumors arise. They arise in the same tissues and in the same organs as the tumors of man; they follow the same clinical course; they cause death in the same ways. Under the microscope they present the same appearance as similar tumors in similar organs in man. *They are the same biologic entity as similar tumors in similar organs in man. And consequently, if we do not discard the theory of evolution, we must admit that they behave in the same way in the matter of heredity as in all other matters.*

When we have found, as we have found in this laboratory for twelve years, that carcinoma and non-carcinoma tendencies segregate out and are transmitted as such; that sarcoma and

non-sarcoma tendencies segregate out and are transmitted as such; that a specificity of tissue type in specific organs segregates out and is transmitted as such, so that an organ either has or lacks the non-cancer mechanism; *when, I say, we have found that these unit characters segregate out and are transmitted as such in mice, so that we can analyze individuals and manipulate these unit characters in heredity as you can pour HCl on Zn with a known outcome—unless we discard the entire biologic science of today, we must admit that these same unit characters segregate out and are transmitted as such in man.*

Moreover, the human statistical evidence admitted by the most vigorous opponent of cancer heredity, itself demonstrates the inheritability of cancer in man, when it is correctly and biologically read.

SUMMARY

1. Cancer and non-cancer tendencies segregate out and are transmitted as such.

2. They are therefore unit characters.

3. A specificity of tissue type in specific organs from ancestor to offspring segregates out and is transmitted as such.

4. It is therefore a unit character.

5. Since these things are unit characters, it is possible to manipulate them by selective breeding and thereby to implant them indelibly in any species, *or to eliminate them permanently and completely from any species.*

6. Cancer and non-cancer behave like the absence and presence respectively of a mechanism fitted to control proliferation and differentiation in regenerative processes, and an animal either has this mechanism or lacks it, no matter to what species he may belong.

7. There is therefore a ready and certain genetic method of escape from cancer for the individual and for the race.

8. The demonstration of the inheritability of cancer and non-cancer tendencies in mice is a demonstration of the inheritability of these tendencies in man and in all other species which show cancer, if we are to maintain the theory of evolution and to admit that there is such a thing as biologic law.

9. The study of cancer behavior, which has demonstrated itself to be fundamentally a biologic problem, makes evident the necessity of understanding and considering the biologic facts underlying all pathologic conditions.

10. And, therefore, when we have got biology under all our pathology and bacteriology, all our physiology and therapy, there will no longer be these monstrous diseases, but only the slow and natural death which is the fatigue and diminution and final cessation of the organ and the organism.

11. From the procedure of analyzing stock into its unit characters in order to manipulate the cancer tendency, there has emerged the fundamental law of heredity—What goes into the germ plasm must come out in the offspring.

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ACIDOSIS, ALKALOSIS, AND TUMOR GROWTH

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The work of Henderson (1) and others has shown that the blood maintains its acid-base equilibrium by balancing carbonic acid against the carbonates, assisted to a minor degree by other acids and bases of the body, notably the phosphates and the proteins.

The presence of an excess of acid in the circulating blood causes an immediate fall in the bicarbonate content, which is soon reflected in the body at large. If the quantity of acid be so large that it cannot be buffered, acids accumulate in the cells throughout the organism and cause a diminution in oxidation rate, the appearance of autolytic enzymes, and a general slowing down of all vital processes (2).

The frequent regression of transplanted tumors and the presence of widespread necrosis in them suggest that many of their cells are, after all, living a sort of hand-to-mouth existence; and a diminution of protoplasmic buffers, so vital a matter to the normal element, might conceivably suffice to turn the scale against the cancer cell.

Accordingly, hydrochloric acid of a strength of from $N/10$ to $N/5$ was daily given subcutaneously at a distance from the tumor, and in doses of from 0.10 to 0.50 cc., to a large number of mice with young transplanted neoplasms. It was realized that even $N/10$ acid would soon cause ulceration, but the difficulty of administering fluids orally to mice left no other course open. Ulceration was avoided as much as possible by varying the injection site.

The dose employed was sufficient to cause a loss of about 1 gram each week in mice averaging 17 grams at the beginning of

the experiment, and corresponds relatively with the amount which Householder (3) found fatal to guinea-pigs within three weeks. Calculated from the therapeutic dose in man, a safe daily dose for a mouse would be in the neighborhood of 0.05 cc. of N/10 acid.

In spite of the large amounts of acid administered, the tumors steadily increased in size, even though the experiments were continued in some cases for three weeks. It is true that growth was sometimes retarded, but this is of not the slightest consequence, for it is well known that proliferation is slower in sick animals.

Enormous doses of sodium bicarbonate were equally ineffective; in fact, proliferation seemed perhaps more rapid in the treated mice.

The experiments demonstrate once more, though no such proof is needed, the marvelously effective adjustment of the tumor cell to its environment.

SUMMARY

Neither acidosis nor alkalosis has any effect upon the growth of transplanted mouse tumors.

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FIBROSARCOMA OF THE SKIN IN A GOLD FISH
(CARASSUS AURATUS)

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The fish, a gold carp, was obtained alive from a garden-pool of a hotel at St. Augustine, Florida; it died during transport, so that the study of the tumors was necessarily confined to a purely anatomical investigation. The pool contained very strongly sulphurous water, and was lined with rough, jagged rocks. The fish had lived in it for at least fifteen years; there were 4 other gold carp in the pool, all of which were apparently healthy.

GROSS DESCRIPTION

Female gold carp, 27 cm. in length, fully developed and well nourished. There are 3 tumors on the right side of the body all having broken through the epiderm (fig. 1). The largest is situated just laterally and somewhat anteriorly to the cephalic border of the dorsal fin. It is moderately firm, round in shape, measures 26 mm. in diameter and projects 15 mm. above the surface of the skin. In appearance it is dull white, its surface is generally smooth, but here and there are superficial ulcerations. At the periphery there is a number of fringe-like projections which extend like buttresses into the neighboring superficial tissue. The cut surface is dull white and almost homogeneous. In places, however, the tissue seems to be gathered into bundles which have a whorled arrangement. The bulk of the tumor is definitely external to the corium, but a considerable portion extends into the musculature and the tissues about the vertebral column. From gross examination it is not only impossible to decide its origin, but it is also difficult to judge as to

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Fig. 1. GOLD CARP WITH CUTANEOUS FIBROSARCOMA

The larger nodule is probably the primary neoplasm; the two smaller tumors are looked upon as metastatic growths

whether the growth arises in the deep tissue and extends outwards, or whether the reverse is the case.

The second tumor has an irregular, somewhat star-like appearance, and lies a little anteriorly to the caudal border of the dorsal fin, and 1 cm. from the medial line. It projects 4 mm. above the surface and has an approximate diameter of 10 mm. Its external and internal appearance is like that of the larger growth, except that it is everyhere definitely external to the corium to which it is attached.

The third tumor is a little round nodule 3 mm. in diameter, about 1 cm. caudal to the second growth. It has the same general character as the second neoplasm.

The internal organs were examined grossly; no tumors were found. Portions of the tissues were fixed in formalin and stained with both haematoxylin and eosin, and Van Gieson's picro-fuchsin; other portions were fixed in Zenker's fluid and stained with Mallory's phosphotungstic acid haematoxylin, anilin blue, and eosin-methylene-blue.

HISTOLOGICAL DESCRIPTION

Largest tumor

The growth lies partly external, partly internal to the corium. The latter, however, is everywhere infiltrated by tumor cells, and in addition there is a moderate infiltration with small round cells, indicating probably a slight reaction on the part of the tissue. The corium is moderately compact at the periphery of the tumor, but its layers are more and more split and finally disappear entirely towards the more central parts of the neoplasm.

The growth is composed almost entirely of very loosely arranged spindle-shaped cells, which are frequently gathered into interlacing bundles, so that in the section the cells are cut in all axes (fig. 2). They are fairly large and resemble moderately mature fibroblasts. The cytoplasm is fairly abundant; at the points of the spindles it generally feathers out into fine fibrils which take the connective tissue stains, and communicate with similar fibrils of neighboring cells (fig. 4). The nuclei are

FIG. 2. FIBROSARCOMA OF GOLD FISH

Low power photograph showing the general character of the tumor. The cells are the connective tissue series, and are gathered into interlacing bundles.

FIG. 3. FIBROSARCOMA OF GOLD FISH

Higher power photograph of portion of preceding figure, to show spindle-shaped character of the tumor cells and their loose arrangement.

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FIG. 4. FIBROSARCOMA OF GOLD FISH

The drawing shows the delicate fibrils at the points of the cytoplasmic bodies of the tumor cells.
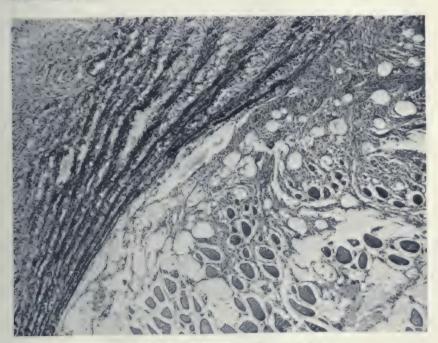
FIG. 5. FIBROSARCOMA OF GOLD FISH

The photograph shows the infiltrating character of the tumor. The corium is split, and invaded by neoplastic cells. The infiltration and destruction of the muscle tissue is apparent in the lower half of the photograph.

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prominent and somewhat vesicular; the nuclear membranes are distinct; one or several dark staining nucleoli are usually present. Where the corium has been destroyed the tumor cells infiltrate the subjacent muscle tissue, which is distinctly atrophic and occasionally entirely replaced by the new growth (fig. 5).

Besides the cell type described there are a few large mononuclear elements with pale vacuolated cytoplasm and poorly staining round nuclei. In many portions of the growth, but especially where it is actively infiltrating, many irregular mitotic figures are seen.

The blood supply is extremely scanty; the vessels in all of the sections are very thin walled, consisting as a rule of a single layer of endothelial cells. Occasionally small irregular hemorrhages are found.

The smaller tumors have the same general cellular structure, but they do not penetrate the corium.

Search for parasites and particularly myxosporidiae was unsuccessful. In all probability the growth is therefore a true neoplasm. Its general histological appearance is that of a very loosely arranged, moderately malignant fibrosarcoma.

DISCUSSION OF SPECIMEN

Analysis of the tumor shows one, the larger, to be infiltrating into the deeper tissues, while the others are external to the corium to which they are attached. The two smaller tumors may probably be looked upon as metastatic growths. This is of interest since, as will be pointed out below, the occurrence of metastasis is very rare in fish. The fibrous corium was probably the primary seat of the neoplasm. It is not possible to do more than speculate as to the causative factors, but it seems not unlikely that the fish sustained frequent cutaneous injuries on the rough rocks which lined its pool, and its advanced age would also favor neoplastic growth.

DISCUSSION OF FISH TUMORS IN GENERAL

Until 1900 only about a dozen instances of neoplastic disease in cold blooded animals had been reported, but since then a

considerable number of fish tumors has been investigated. This is partly due to the stimulation given by the researches in this field of Pick (1), Plehn (2), Fiebiger (3), Murray (4), Bashford (5), Schmey (6), Gaylord and Marsh (7), et al., and partly to the ever increasing economic importance of fish, necessitating more thorough knowledge of their diseases. The review of the literature by Schmey (6), and one more recent by Fölger (8), bring to light certain facts of general interest. Schmey found (in 1911) 59 reported cases of true neoplasm; these he tabulated as to type of tumor, organ involved, and species or genus affected. His tables show that almost all of the more important forms of benign and malignant tumors may occur in fishes. Thus there have been observed; osteoma, fibroma, lipoma, angioma, myoma, various forms of sarcoma, endothelioma, adenoma, papilloma, both "benign" and malignant epithelioma, and carcinoma. The thyroid, cutaneous surfaces and their appendages, musculature, intestinal tract, peritoneal cavity, urinary bladder, air bladder, oral mucosa, liver, and kidney have been recorded as the seat of the new growths.

The distribution is curious. Up to the present time, tumors have been found only in teleosts (bony fishes, to which most living forms belong), and particularly in the order Physostomi (the older classification of Hertwig, Lehrbuch der Zoologie, Jena 1909, has been followed). Two families belonging to this order, the salmonoid and ciprinoid fishes, have furnished the greatest number of cases. These two families are furthermore of special importance since they furnish examples of neoplasms confined to a particular group of fish, in which they are apt to occur endemically or epidemically. Probably the most intensively investigated form is the thyroid cancer of the salmonoids, popularly known as "throat tumor," or "gill disease." This disease has been known for about thirty years, and manifests itself as a rapidly growing tumor on the floor of the mouth and on the gills, which usually terminates fatally. It has been studied on a large scale by Gaylord and Marsh (7), in whose monograph (1914) it is shown that while most of the tumors have been found in domesticated fish (from fish breeding stations, etc.), they have

been observed also in fish caught in their native condition.   There can be no doubt that the growth represents a true carcinoma, nor that a living organism is its causative factor, but no evidence has been found to indicate the direct transmission from individual to individual, except that epidemics break out in certain localities and thus make its transmissable nature probable. Gaylord and Marsh were able to induce analagous tumor growth in mammals by administering with drinking water the scrapings from the inner surfaces of wooden troughs which had harbored tumor fish.   The tumor-producing substance was destroyed by boiling.   Fish in all stages of the disease were favorably affected in the direction of cure by the addition to the water supply of mercury, iodine, or arsenic in suitable concentrations. Spontaneous recovery also occurred in a considerable percentage of individuals, and some strains appeared immune to the disease. The great importance of this research for comparative oncology as well as for practical fish culture is apparent.

The other most widely spread form of tumor is the so-called "pox" of carps.   It, like the thyroid cancer of the salmonoids, is apparently confined to a single family, the cyprinidae (carp family).   The condition manifests itself as an epithelial hyperplasia; it begins as milky opaque cutaneous plaques, which gradually become thicker and project for several millimeters above the skin surface.   They are usually tough, like cartilage, and readily detached from the subjacent corium.   The cause of the "pox" is a disputed subject.   The early observers (Hofer (9), Döflein (10)) believed the growth to be parasitic in origin, but they were unsuccessful in demonstrating either vegetable or animal parasites within the lesions.   Later, these investigators held that the skin lesions were due to parasites, but indirectly, since within the kidney of the affected carp, myxosporidiae (Myxobolus cyprini Hofer) could be found practically always.   It was argued that the myxobolus infection so lowered the functional capacity of the kidneys that metabolic products otherwise eliminated by them were now vicariously excreted through the skin, and that either through constant irritation or through hyperactivity, a cutaneous hyperplasia resulted.   It was, however,

shown by Plehn (2), Fiebiger (3), and others that myxospori-
diosis occurred quite commonly in carp not affected with "pox,"
and that, on the other hand, some fish with "pox" did not con-
tain the parasites in any of their internal organs. At present
the majority of observers, therefore, hold that the theory of
Hofer and Döflein is not supported, and that the cause of the
disease is still unknown. There is also some disagreement as
to the nature of the process. Plehn looks upon it as a be-
nign hyperplasia, which only occasionally becomes infiltrative
and malignant. Fiebiger is inclined to believe that under the
term "pox" or "benign epithelioma" several very different
conditions have been described, and that the epidemic "pox"
is nothing more than an inflammatory overgrowth of the epiderm.

As to other tumors, we find a great variety of types in both sea-
fishes and fresh water fishes. Their histological structure is
quite comparable to that of mammalian neoplasms, but there
are certain outstanding differences in their behavior. Thus the
occurrence of metastasis is rare, even in growths which tend to
destroy life and which histologically have an infiltrative and
locally destructive character. Gaylord and Marsh, in their
extensive series of thyroid cancers, observed only a single in-
stance of unquestionable metastasis to internal organs, and only
a few examples of doubtful metastasis. Similarly Schmey, in
his compilation, records only two metastasizing tumors, both
sarcomata. The epithelioma of a catfish reported by McFar-
land (14) and the fibro-sarcoma described here are the only
other examples of true metastatic growth which we have been
able to find recorded. At the present time, no explanation can
be offered for this rarity of metastatic dissemination.

The neoplastic nature of a fish tumor is not always easily
recognized. Murray (4) emphasized particularly the difficulties
which confront the pathologist when studying sarcoma-like
growths, since our knowledge of inflammatory reaction in cold
blooded animals (and indeed in most animals) is as yet very
scanty.

Kitt (11) states that in fish the skin is the most frequent seat
of neoplasms, and explains this on the ground that the cutaneous
surfaces are most liable to frequent injury. This statement

is borne out by a review of the literature only if the numerically very frequent "pox" of the cyprinoid family is included. Fiebiger, in his paper on the skin tumor of fishes, gives a very good account of the normal histology of the cutaneous structures and reports several epitheliomata, a fibroma, and a multiple papilloma. An osteoma and a sarcoma have been noted by Schröder (12), carcinoma in a carp by Bashford, and a carcinoma in a gold fish by Dauwe and Pennemann (13).

McFarland (14) was probably the first to study cutaneous epithelioma in the fish; his report occurred in the transactions of a pathological society and seems hitherto to have been overlooked. The tumor, an epithelioma of the mouth and skin of a white catfish (caught in Pennsylvania), is of further interest in that probably it is an example of true metastasis. A number of tumors were present; the largest arose from the lower jaw and projected into the mouth, probably interfering with its closure. It measured almost 4 x 2.5 x 1.75 cm., was papillary, greyish-white, and had an ulcerated surface. Numerous nodules occurred along the edge of the upper jaw; others about the eyes and the inferior surface of the neck; these nodules appeared to be purely dermal, and probably secondary to the growth in the lower jaw. There were no tumors in the gills, upon the body, or in internal organs. Histologically the neoplasm was an infiltrating papillary squamous epithelioma, arising probably from the oral mucosa.

Lastly, as to tumors hitherto described in goldfish, we are able to find but two instances in the literature, a skin cancer, reported by Dauwe and Pennemann, and a cancer of the urinary bladder described by Plehn. This is the more surprising since hybrids and domesticated fishes appear to suffer more commonly from neoplastic diseases than pure or wild species. It may be, of course, that tumors in this species have not been brought to the attention of pathologists, and that more extensive investigation would bring to light further instances. Since the gold fish is almost entirely an aquarium specimen, thoroughly domesticated and used to room temperature, it would seem to be ideal for experimental tumor studies, and it is hoped that the present report may draw attention to this problem.

SUMMARY

1. A fibrosarcoma of the skin of a gold carp is reported.

2. This tumor gave rise to metastatic growths, a rare occurrence in fishes.

3. As far as we are able to learn this is only the third instance of neoplastic disease reported in goldfish.

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THE ACTION OF BURIED TUBES OF RADIUM EMANATION ON NEOPLASIAS IN PLANTS

ISAAC LEVIN AND MICHAEL LEVINE

From the Cancer Division of the Montefiore Hospital for Chronic Diseases, Dr. Isaac Levin, Chief

The insertion into an animal tumor of buried capillary glass tubes containing radium emanation presents a comparatively new departure in radium therapy and is undoubtedly destined to play a prominent part in the future development of the whole field of radiotherapeutics. The technique employed on animals and human beings is as follows: Radium emanation, the first active product of decomposition of radium, is an elementary body in a state of a heavy gas, and by means of appropriate apparatus may be collected in capillary glass tubes 3 to 5 mm. long, and 0.25 mm. in diameter. These tubes may be made to contain anywhere between 0.1 to several millicuries of radium emanation each. They are inserted into the tumor tissue by aid of a trocar.

The tubes exert a comparatively weak but continuous action on the tissues which lasts for several weeks. The cumulative action of 1 mc. when buried permanently in the tissue is calculated to equal 132 mc. hours. The tissue immediately surrounding the capillary is influenced by the soft beta rays and may become necrotic. Figure 1, *A*, shows such an area of necrosis in the spleen of a rabbit into which a radium emanation capillary was inserted. A priori it is feasible to anticipate that this necrotic area acts as a filter on the soft rays and the next zone of tissue is then influenced only by the hard gamma rays of radium.

In the course of the last two and a half years, the senior writer has used this method extensively in his clinical work. As a general rule the insertion of capillary glass tubes in carcinoma

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and sarcoma in the human patient is followed by the replacement of the tumor tissue, in the vicinity of each capillary tube, by a connective tissue capsule which wholly encloses the tube. The whole tumor shrinks in size, a process which takes place gradually in the course of six to eight weeks after the insertion of the capillary tubes.

The clinical results to date appeared to be of such importance that it seemed imperative to investigate biologically the mechanism of the action of this method of radium therapy.

In a previous study on the influence of x-rays on the development of the crown gall, the writers (1) have shown that neoplasia of plants presents an ideal tissue for the study of the subject since the results of the action of the rays on the plant tumor cells are not obscured in plants by blood, lymph, and the connective tissue, as is the case in animal tumors. This study of the action of the x-rays on the crown gall indicated that the main immediate action of the rays consists, not in a direct destruction of the cells, but in the arrest of their proliferating power. The death of the cells follows as a consequence of the aging of the individual tumor cell.

The present investigation consists in the application of the method of insertion of buried capillary glass tubes filled with radium emanation in plant tissue, and in a gross and a microscopical study of the resulting changes.

Adult normal plant tissue, and particularly young growing tips of plants, were used for purposes of comparison, while crown gall and club root tissues were the main materials.

MATERIALS AND METHODS

The normal tissues used in our experiments consisted of young and adult roots of the purple-top turnip and the growing tips of the tobacco plant. Club roots on cabbage and kohlrabi, artificially produced, and crown galls on the geranium were the main material. Capillary tubes 3 mm. long and 0.25 mm. in diameter containing radium emanation were introduced into the plant. This was done by making a pin-hole opening in the desired part of the plant by means of a sterile needle and then introducing the sealed tube containing the emanation.

The tube of radium emanation was left buried in the tissue for from one to fifteen days and the plants were observed carefully at regular intervals. Empty tubes equal in size to those containing the emanation were inserted in identical tissues as controls. The irradiated and non-irradiated tissues were killed in a variety of fixing agents, of which Flemming's strongest solution and Cornoy's fixative gave the best results.

The material was then cut in sections 5 to 7.5 microns thick. They were stained with iron hematoxylin and Flemming's triple stain.

<div align="center">OBSERVATIONS</div>

The effect of the insertion of a tube of radium emanation into young root tissue is shown in figure 1. This figure represents a longitudinal section of the root through the region where the tube was inserted. The tube contained 2.2 mc. of radium emanation and the photograph was made fourteen days after the needle was buried in the tissue.

The dark area represents the area of necrosis surrounding the tube for a distance of 2 to 3 mm.

A control, empty tube was inserted into the root of a young turnip plant fourteen days previously, growing in the same plot, is shown in figure 2. The tube may be seen in place and the necrotic area here is represented by a small narrow line where the sterile needle made an opening to permit the insertion of the tube. In mature roots, similar results were obtained.

Figure 4, A, and 4, B, represents cross sections of two purpletop turnips growing side by side. The plant shown in 4, A, received a glass tube containing 3.4 mc. of radium emanation while 4, B, received only a sterile glass tube. Figure 4, A, shows a necrotized area surrounding the radium tube while 4, B, shows a narrow line representing the region where the empty glass tube was inserted, fourteen days previously, similar to our observations made in young roots.

The effect of a tube of radium emanation 1.8 mc. after being inserted into the growing tip of a mature tobacco plant, is shown in figure 5, A. The tube was inserted at the time this branch

was beginning to form flower buds, just below the tip of the branch but still in its growing region.

Another branch of the same plant shown in figure 5, B, in the same stage of development, received an empty capillary tube in the same region of the branch. No interference with the growth of the flower stalk or buds is visible here.

In the case of the first branch which received the radium emanation, a change was noted twenty-four hours after insertion of the tube. A small necrotic area appeared forty-eight hours afterward, and this blackened area around the tube grew larger and larger until it cut off the tip of the plant, or the part above the necrotic area. The part below the necrotic area showed no visible effect. Similar changes were noted in the growing tips in young plants before flowering stalks had developed. Similar results with quantities of radium emanation varying from 0.3 to 3 mc. were also noted. With the larger quantities of radium emanation, however, the necrotic areas, it appears, were more readily noticeable after a relatively short period.

THE EFFECT OF RADIUM EMANATION ON CROWN GALL TISSUE

The study of the effect of radium emanation on crown-gall tissue was done on the geranium. Inoculation of the apical part of the geranium stem with Bacterium tumefaciens was followed by the insertion of a tube of radium emanation one to thirty days after the inoculation was made.

A large number of geranium plants grown during the same period were matched for size and put in pairs. One of a pair received a tube of radium emanation in the region of inoculation, while the other served as a control and received an empty tube.

Figure 6 represents two plants growing in 6-inch pots in a green house under similar conditions. Both were inoculated at the same time with a culture of Bacterium tumefaciens. *A* received an empty tube in the region of inoculation,—two days after the inoculation was made. *B* received a tube containing 0.4 mc. radium emanation. Very early in the study of these plants, of which these were two of a series of thirty such experiments, *A* showed a slight swelling, a week after the inocu-

lation with the Bacterium was made, while B showed very early a blackened area around the tube and no swelling.

When the photograph (shown in fig. 6) was taken, two weeks after inoculation and twelve days after the application of the radium emanation, A showed a recognizable crown gall, while B showed a black necrotized area surrounding the tube and no evidence of crown gall. In all these experiments similar results were obtained.

Microscopic studies of small crown galls approximately one month after infection, which were irradiated with tubes of radium emanation, were studied from one to fifteen days after the radium had exerted its influence on the neoplasm. In the early stages after the insertion of the tube is made into the crown gall, the effect becomes noticeable two to three days later. Examination of sections under the microscope shows that the tissue surrounding the tube is necrotized and drawn away from the glass tube; the radial cell walls have collapsed so as to form a more or less compact layer of cell walls or cellulose around the glass tube; the cells immediately surrounding the cellulose capsule have disintegrated; and one occasionally finds the nuclei and cytoplasm in the process of disintegration, while the layer of cells beyond this zone is apparently unaffected. The first zone or cellulose capsule is characteristic of the effects of the radium. While the disintegrating zone is present, it blends with the outer zone, which is not visibly affected.

Figure 7 represents a microscopic section of a young crown gall into which a tube of radium emanation was inserted for five days. The cellulose capsule is markedly visible in B. The plasmolized and disintegrating cells are visible in the enlargement shown in figure 8, C. Normal nucleated cells are visible in the area beyond C.

THE CLUB ROOT

Club roots artificially produced by infusion inoculations in cabbage and kohlrabi roots were also tested with tubes of radium emanation. Figure 9 represents the thickened finger-like roots of a young kohlrabi plant into which a tube containing 0.4 mc.

of radium emanation was inserted. As in the case of the irradiated crown-gall and normal tissues, a small necrotic area appeared around the edge of the tube several days after its insertion.

Microscopic sections of the root are shown in figure 10. A marks the region where the tube was inserted. The cellulose cushion is here visible at B. The xylem tubes shown to the left have not collapsed. The hypertrophied parasitized cells are not markedly affected but the hyperplastic tissues are devoid of cytoplasm and nuclei. In general, the same appearance is presented here that was observed in the case of the irradiated crown galls.

DISCUSSION

In the normal adult tissue the only perceptible result of an insertion of a radium emanation tube is complete destruction of tissue in the immediate vicinity of the capillary. The tissue beyond this area does not seem to be influenced in any way which corresponds with results obtained in animal tissue. Adult tissue is not affected by moderate amounts of gamma radiation.

Fig. 1. Microscopic section of the spleen of rabbit showing the effect of buried radium emanation tube.

Fig. 2. Young root of turnip showing effect of buried radium emanation tube.

Fig. 3. Young root of turnip showing effect of buried empty tube.

Fig. 4. Cross section of mature root of turnip. A, received tube of radium emanation; B, control, received empty tube.

Fig. 5. Shows the effect of buried radium emanation tube in the growing point of a tobacco plant, A, and of control empty tube buried in B.

Fig. 6. Shows the effect of buried radium emanation twenty-four hours after the geranium was inoculated with bacterium tumefaciens. A, control; B, received tube of radium emanation.

Fig. 7. Microscopic section of a crown gall in which a tube of radium emanation was buried. A, region tube occupied; B, cellulose capsule; C, disintegrating cells.

Fig. 8. Microscopic section of same part, position of crown gall, higher magnification.

Fig. 9. View of root of young kohlrabi plant infected with club root organism showing buried radium emanation tube.

Fig. 10. Microscopic section of club root; A, region of tube.

The insertion of radium emanation tubes into the crown gall tissue is followed by an inhibition of the development of the neoplasia which is evidenced by the reduction of the size of the tumor as compared with the controls. This is an indication of the inhibition of the nuclear proliferating activity of the gamma rays of radium on the tumor cells.

The tumor tissue in the immediate vicinity of the buried radium emanation tubes is affected mainly by the soft beta rays. Here, therefore, deeper changes take place in the tumor tissue. Sections of this region show the collapse of cell walls radially to the capillary tube, forming a cushion of cellulose. The cells immediately behind this cushion are devoid of both nucleus and cytoplasm. Occasionally one finds a nucleus in process of disintegration. This disintegrated tissue and the cellulose cushion filters off the soft gamma rays. Cells further back of this area are consequently acted on only by the gamma rays.

As was shown in previous studies of I. Levin and M. Levine (2), and I. Levin and B. Joseph (3), such action of gamma rays may not be followed by evident morphological changes in the tumor cells. None the less, this proliferating power will be inhibited, and the tumor will not increase in size.

It is significant that the cellulose cushion seems to play a rôle in plants, in walling off the necrotic area about the radium emanation tubes and filtering off the soft beta rays, similar to that played by the connective tissue stroma in animal tumors.

In club root tissue the degenerated cells immediately adjoining the so-called cellulose cushion do not seem to contain the Plasmodiophora brassicae, while the parasite is present in the cells at a distance further from the capillary tube.

This apparent action of radium on the parasite as well as the more minute study of the intracellular changes caused by the irradiation is a subject of further study by the writers and will be reported later.

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THE RÔLE OF NEOPLASIA IN PARASITIC DISEASES OF PLANTS

ISAAC LEVIN AND MICHAEL LEVINE

From the Cancer Division of the Montefiore Hospital for Chronic Diseases, Dr. Isaac Levin, Chief

In a previous investigation (1) on the malignancy of the crown-gall and its analogy to animal cancer, the writers have demonstrated that a crown gall does not develop through the specific neoplastic properties of the Bacterium tumefaciens. The mechanism of the formation of these tumor-like structures takes place in the following manner: The invasion of a plant or its inoculation by Bacterium tumefaciens is followed by an attempt of the host at self protection. Since a plant organism lacks blood, lymph, and lymphoid tissue, this protective mechanism cannot attain the character of an inflammatory process with subsequent scar formation. Therefore, in plants this protective mechanism must be supplied by the life functions of all the tissue cells in the neighborhood of the invasion of the parasite. That this life function of the tissue cells would consist in their active proliferation and in the undifferentiation of the newly created young cells is a priori more than plausible. Evidence was brought forward in the previous investigation to support this view.

In the present publication further studies are reported on the mechanism of the formation of neoplasia in plants subsequent to their invasion by parasites.

A striking instance of undifferentiation of newly formed cells for purposes of self-protection is presented by the junior writer (2) in his study on the mechanism of the formation of the leafy crown gall, in which he adduced evidence to show that Bacterium tumefaciens does not cause the formation of leafy shoots in Bryophyllum calicynum but rather inhibits and retards their

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normal development, when inoculated into the totipotent cells which appear at the notches of the leaf. The following is in brief the method of investigation employed: When leaves of Bryophyllum are detached from the mother plant and put on moist soil, the marginal notches of the leaves at which totipotent cells are found develop into leafy shoots and eventually form new plants. In this study the junior writer inoculated notches of one side of the leaf, by pricking the tissue five to ten times with a delicate needle containing a culture of Bacterium tumefaciens. The inoculated notches of the opposite side of the leaf served as controls, after they had been pricked with a sterile needle. As a result of the experiment the notches infected with Bacterium tumefaciens in the great majority of cases, instead of developing leafy shoots formed ordinary crown galls (figure 1, A), while the control notches pricked with a sterile needle all developed leafy shoots (figure 1, B). Occasionally a poorly developed shoot appears over a gall.

The significance of this investigation consists in the following: The totipotent or embryonic developmental cells of the notches proliferate to a certain extent, then differentiate into new organisms. When parasites invade the region of these cells the latter do not differentiate at all, or differentiate incompletely, and their main function then consists in creating a multitude of young undifferentiated cells. Thus a neoplasia—"gall" —is formed, which is evidently an attempt at a protective reaction.

In their publication cited above, the writers have shown that a benign gall is analogous to a granuloma, which is the protective mechanism in an animal organism, while a malignant crown-gall is analogous to animal cancer.

In a later investigation the junior writer (3) has shown that the behavior of even a malignant crown gall differs greatly from the mechanism of malignancy in animal cancer.

The research was done on the rubber plant (Ficus elastica), an evergreen perennial plant on which the course of the development of the crown gall can be watched for a longer period of time than on annual, biennial, or deciduous trees.

This investigation shows that a few months after inoculation with the Bacterium tumefaciens, the majority of the crown gall remain benign but later many of the galls become malignant and destroy the inoculated branch.   Figure 2 shows a large benign crown gall seven months after inoculation, and figure 3 illustrates the same crown gall six months later when it became malignant and is destroying its host, the branch of the plant.   The gross and microscopical analysis of this branch shows the following condition.   The crown gall itself is dead but it shows microscopically a differentiation of tissue which converted the crown-gall cells into a mass of parenchymatous cells and nodules of woody fibers.   The major portion of the stem of the branch above the gall is dead, with the exception of the top of the stem which is still green.   A part of the stem below the gall is also dead.

The subsequent differentiation of crown gall tissue was noted by the writers in their previous publication.   In animals such a phenomenon never occurs in cancer, but only in protective reactive neoplasia, as in the formation of a scar from a granuloma or the formation of a protective connective tissue stroma around a malignant tumor.   The necrosis of the crown gall and surrounding stem tissue also differs radically from malignancy in animal cancer, in which the destruction of vital organs is always caused by invasion of living active cancer cells. Kunkel, in his studies on club root of crucifers, ascribes the wilting of the infected plants to the fact that the ever increasing number of transpiring cells (green parts of the plant) grows too fast for the atrophied development that takes place in the conducting system.. Possibly a similar indirect mechanism may be the cause of all malignancy in plant neoplasias.

In order to obtain additional information in regard to the cause and mechanism of the formation of neoplasia of plants, two additional parasitic diseases of plants were studied by the writers, namely, the club root of cabbage and the potato wart.

CLUB ROOT OF CABBAGE

Our studies on club root were confined to the inoculation of roots with Plasmodiophora brassicae made by the infusion method. Old club roots were soaked in water for from five to six days and the infusion was then poured over the roots of young plants grown in pots and in our experimental garden at Montefiore Hospital. When infection had occurred and the resulting hypertrophies or hyperplasias were obtained, the plants were removed from the soil. Small pieces were fixed in Flemming's solution for forty-eight hours and then sectioned and stained by Flemming's triple method.

Figure 4 represents a young cabbage plant, the small secondary roots of which are very much thickened due to infection with the club root organism. Figure 5 represents a high magnification of a microscopic section through such an infected root. The dark colored cells contain the spores of the infecting organism which are liberated when decay sets in. Figure 6 shows at A areas of small young undifferentiated cells which are not parasitized and surround the large hypertrophied and parasitized cells.

Club root is a plant tumor similar to the crown-gall in its derivation, mechanism of formation, and effect on the host plant. The advantage in the microscopical study of the club root over the crown gall consists in the fact that the Plasmodiophora brassicae is easily observed within the cell while the Bacterium tumefaciens cannot be detected. The most striking phenomenon observed on microscopical study of the club root is the fact that the groups of large cells containing the parasites are always surrounded by layers of small young cells which do not contain the parasite. Kunkel (4) maintains that the growth stimulus travels in advance of infection; that is, the parasite had no time to infect these cells. Another explanation given by him is that the noninfected cells are immune to the parasite. The most plausible explanation is that these young undifferentiated cells are not only immune to the parasite but present a reactive protective barrier against further inroads of the latter. That

this neoplastic barrier may not be efficient and that ultimately
the parasites may break through does not in the least vitiate
the hypothesis.

<div align="center">POTATO WART</div>

The potato wart disease, which is a relatively new disease in
America, has long been known in Europe. The government
restrictions placed on transportation or cultivation of potatoes
bearing this disease made it impossible for us to secure potato
plants with this disease so that we could actually grow them as
we did with the crown gall and club root. However, through
the courtesy of Profs. F. D. Kern and C. R. Orton we were able
to make our preliminary studies on old potato warts fixed by
them, and group this disease with the neoplastic diseases de-
scribed above. Recently, Dr. Freeman Weiss has been kind
enough to give us blocked material, also several fixings of potato
warts from plants growing in his green house at the United
States Department of Agriculture.

The authors take this opportunity to thank Drs. Kern, Orton,
and Weiss for their courteous coöperation.

Figure 7 is a reproduction of a plate published by Kern and
Orton (5) showing the appearance of the potato when affected
by the organism, which is probably a Synchytrium, as held by
Percival, and later by Miss Curtis. The wart tissue comes from
modified and stimulated epidermal cells of the potato, which
are the cells to be first invaded by the parasite. This neoplastic
tissue finally covers the whole potato and interferes with the
normal development of the potato tuber.
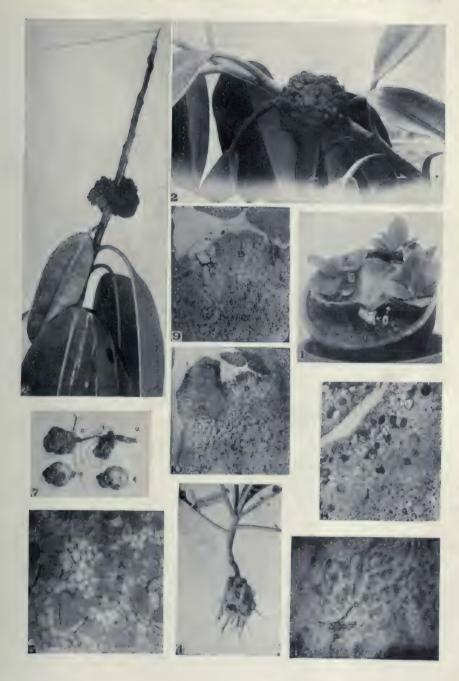
Figure 8 represents a section through a wart in which the
parasites can be seen in abundance. Large sori with sporangia
filled with spores are most common. The resting spore also
appears in the center of the field. The neoplastic tissue, which
consists of young undifferentiated cells, may be seen surrounding
the parasitized cells. These cells are small, containing a sharply
differentiated nucleus, and a dense cytoplasmic structure in
which no starch grains are visible. Below this area of small
undifferentiated cells are the normal potato cells, larger in size

and filled with starch grains. The early stages of development of the wart may be seen in figures 9 and 10. These are especially clear as to the neoplastic tissue and the section may be divided into two parts. The outer, or epidermal parts contain the potato cells infected with the parasite and a correspondingly large number of small rapidly dividing cells which are uninucleated and apparently unparasitized.

Gross and microscopic analysis of the material shows substantially the same relationship between the parasite, the normal adult tissue of the host plant, and the reactive neoplastic tissue as in crown gall and club root of cabbage.

DISCUSSION

1. Neoplasia in parasitic diseases of plants, unlike the neoplasia in animal cancer, always represents a protective reaction of the plant organism against the invasion of a parasite.

2. Unlike malignant neoplasia in the animal, which has no finality and dies only with the death of the whole host organism, plant neoplasia always completes a life cycle. It has a period of progressive proliferation of undifferentiated cells, is frequently

FIG. 1. Bryophyllum leaf with crown galls, A, produced by inoculating the leaf notches with Bacterium tumefaciens; young bryophyllum plants, B, developed from uninoculated buds.

FIG. 2. Crown gall on the rubber plant (Ficus elastica).

FIG. 3. The same crown gall shown in figure 2 six months later.

FIG. 4. Club root on a young cabbage plant.

FIG. 5. Microscopic section of club root under high magnification. Hypertrophied cells, A, filled by the parasite.

FIG. 6. Microscopic section of club root under low magnification showing the distribution of the hypertrophied, A, and hyperplastic tissues.

FIG. 7. A, B, C, D, showing the potatoes in various stages of infection with potato "wart" disease. Taken from Orton and Kern.

FIG. 8. Microscopic section through potato "wart" showing A, hypertrophied cells infected with parasite, B, hyperplastic cells, C, apparently normal cells filled with starch grains.

FIG. 9. Microscopic view of an active young portion of potato "wart."

FIG. 10. Same section under higher magnification.

transformed into adult differentiated tissue, and then regression and death occur before the complete destruction of the host. Thus it behaves more like reactive neoplasia in an animal than like animal cancer.

3. Neoplasia in parasitic diseases of plants never represents a malignant tumor in the true meaning of the term in animal pathology.

4. Neoplastic tissue in plants is constructed of only one type of cells and presents therefore an ideal material for the study of tumor formation.

5. The true relation between the formation of reactive neoplasia and the pathogenesis of malignant tumors in the animal is as yet not established. The relation between infectious granuloma, Hodgkins lymphoma, and lymphosarcoma—a true malignant tumor—presents an instance. The clearing up of this point may aid greatly in the establishment of the true etiology of cancer.

6. The points considered in the previous paragraphs make it evident that the study of neoplasia in plants should become an integral part of all phases of cancer research, whether aiming at the elucidation of the etiology or pathogenesis of the disease or of the correct basis for therapy.

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PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

FIFTEENTH ANNUAL MEETING

Held in Washington, D. C., May 1, 1922

1. REPORT OF THE COUNCIL

The meeting of the Council was held at the New Ebbitt Hotel, on the evening of Sunday, April 30, 1922, at eight-thirty o'clock.

The following members were present: Dr. James B. Murphy, president; Dr. Willy Meyer, vice-president; Dr. Wm. H. Woglom, secretary and treasurer; Dr. H. Gideon Wells. Absent: Dr. Francis C. Wood, Dr. Robert B. Greenough, and Dr. James Ewing.

The report of the treasurer for the year showed a balance on hand of $466.41. The treasurer also reported that the gifts made to the JOURNAL during the past few years by private individuals or various funds had not been entirely expended when the JOURNAL was taken over by the Crocker Fund and that $266.71 of such moneys still remained in the treasury. He asked the members of the Council whether this money should be divided into aliquot parts and returned to the various donors, or should be kept in the treasury. It was decided that it would be justifiable to allow this money to remain the property of the Association, Dr. Wells expressing his opinion that Dr. Ormsby, for example, would rather have his gift used by the Association. It was pointed out, furthermore, that in the case of the contribution from the members of the staff of the Gratwick Laboratory, the amount returned to each would be so small as to be practically negligible. The treasurer was, therefore, instructed to retain this money in the treasury of the Association, and if necessary to expend it for general expenses in connection with meetings, etc.

The name of the following applicant came before the Council for election to the Association:

Dr. Isidor Kross, 20 West 50th Street, New York

Dr. Kross was elected an active member.

The resignations of the following members were accepted:

Dr. G. R. Minot   Dr. J. A. P. Millet

The secretary was instructed to write to Dr. J. P. Hoguet and Dr. Hugh H. Young, who also had sent in their resignations, asking them to reconsider their determination and pointing out that the Association at the present time, more than ever, needed their support.

Dr. Frederick Prime, New York, was elected to the Council to succeed Dr. Francis C. Wood, automatically retired by the time limit.

The following officers were elected for the ensuing year: Dr. Willy Meyer, president; Dr. William Duane, vice-president, Dr. Wm. H. Woglom, secretary and treasurer (re-elected).

The present members of the Council, with the years of their retirement, are:

Dr. James B. Murphy, 1923   Dr. Willy Meyer, 1926
Dr. Wm. H. Woglom, 1924   Dr. James Ewing, 1927
Dr. Robert B. Greenough, 1925 Dr. H. Gideon Wells, 1928
Dr. Frederick Prime, 1929

The present Editorial Board was continued in office:

Editor, Dr. Woglom
Associate Editor, Dr. Prime
Dr. Bloodgood   Dr. Loeb
Dr. Wells   Dr. Ewing
Dr. Tyzzer

It was suggested that the JOURNAL might be made more popular by publishing from time to time reviews of various phases of the cancer problem. The secretary was instructed to write to each member of the Editorial Board to ask for opinions on this proposal.

2. DEMONSTRATION OF GONGYLONEMA CARCINOMA

Dr. Julius Rosenstirn (New York):

SUMMARY

Dr. Rosenstirn stated that in June and July of 1920 he worked for six weeks in the laboratory of the Pathological Institute of Copenhagen, where he made himself familiar with the methods of cockroach experimentation of Dr. Fibiger, who furnished him with a host of cockroaches not infested, with five of the stock of his rats, and an equal number of infested mice, all of which were brought to the Crocker Institute. The life cycle of these nematodes, the intermediate host of which is the cockroach, was described. Either artificially or spontaneously infested mice or rats shed with their feces the ova containing the embryos of the nematode at first classified as spiroptera by Fibiger and Ditlevsen. This classification was later changed by Ransom, who regarded this nematode as belonging to the family Gongylonema, and the correction has been adopted by Fibiger and his collaborator, who since term it Gonglyonema neoplastica. These ova-carrying feces of the infected animals are fed to the cockroaches. Of the three kinds of cockroaches—the Periplaneta americana, Periplaneta orientalis, and the germanica—only the first two, the americana and the orientalis, can be regarded as serviceable for these experiments. The germanica is entirely inadequate, mainly on account of its smallness, and the best, the americana,

seems to be extremely hard to obtain. The experiments under discussion have been carried out exclusively with the orientalis, with which great difficulties were experienced until lately, in consequence of following too closely Fibiger's suggestions. He advised that they be kept at a temperature of 30°C., but at this temperature the cockroaches died. When they were kept at 17° to 20° they did well. The eggs are eaten with the feces by the cockroaches; the embryos are freed in the intestine, and migrate gradually, developing into the larval stage, into the muscular system, where they are encysted somewhat like the Trichinæ. The muscular parts of the infested cockroaches are fed to the rats, and the larvæ lodge in the mucous membrane of the stomach, where they mature to the adult form of the Gongylonema. At times some of the larvæ remain in the tongue and wander from the mucous membrane into the lingual musculature, where they produce the same pathological changes as in the stomach. In a certain percentage of cases carcinoma of the stomach, or rather of the so-called pro-stomach, the cardiac portion of that organ, is caused by the presence of the parasites.

DISCUSSION

Dr. H. Gideon Wells (Chicago): I should like to ask if in Fibiger's experiments there was any instance of carcinoma of the glandular portion of the stomach. It is rather remarkable that in those animals with a rumen, it is not uncommon to find squamous cell carcinoma of the rumen, but never of the glands of the fundus. In carcinoma of the stomach in mice, there are 9 or 10 cases, reported and unreported, in which the carcinoma has always been in the cardia, except in 1 case of a carcinoma of the glandular type. I take it there is some factor of importance here, since carcinoma of the glandular type is the variety seen in man; yet it is exceedingly rare in all the lower animals.

Dr. Erwin Smith (Washington): On the roots of plants destructive tumors due to nematodes (Heterodera radicicola) have been known to botanists for a long time. In these cases, as in the case of the nematode infections obtained by Fibiger in rats, the entire body of the worm is buried in the tumor so that all the excretions of the worm pass into the surrounding host tissues. In this connection it is interesting to know that in Florida there is a nematode on the roots of the orange tree which does not produce any tumors, although Dr. Cobb states that it is closely related to the tumor-producing Heterodera. In this case only the head of the worm is inserted into the tissues, which are sucked dry and killed. The rest of the worm, including the excretory organs, is outside of the root in the soil, and I believe that this is the reason that in the one case tumors are produced, and in the other not produced. Giant cells are very common in the nematode tumors of plants. Often they contain a great many nuclei, and I have seen as many as fifty in a cell. These giant cells, which appear very early in the life of the

tumor (earlier than the sixth day) are, so far as I have observed, always close to the head of the worm, which probably feeds upon their contents. In remoter parts of the tumor there is an hyperplasia. The whole tumor is soft and very perishable.

Dr. E. T. Bell (Minneapolis): I want to call attention to a certain resemblance of this growth to the well known coccidiosis in the liver of rabbits. This is a very common condition; the coccidia grow in the intestines and in the hepatic ducts. There develops an extreme hyperplasia of the epithelium with enormously dilated ducts,—a condition which we might call an adenomatous growth, but never, so far as I know, does malignancy develop. The organisms are embedded in the epithelial cells.

Dr. Rosenstirn: In reply to Dr. Wells, I may say that the cancers develop exclusively in the cardiac portion of the stomach, the so-called pro-stomach, never in the pyloric part, which in man so frequently is the seat of carcinoma. Whether this is due to the different types of glandular structure or of the epithelium in these parts, I do not know, but the fact remains that neither Fibiger nor myself have ever observed these pathological changes outside of the pro-stomach. Concerning the changes produced by nematodes entirely buried in plants, and their absence in the Florida plants, in which only the head lodges and the secretions of the nematodes are poured into the earth, I can not now determine if there is any justification in arguing from the action of these parasites on plants to the action of others on the rat stomach. The secretion of the nematode into the tissues may be an auxiliary factor in producing the hyperplastic and cancerous neoformations. This question, although discussed, has been left open by Fibiger, and its decision appears to offer nearly insurmountable difficulties.

I do not quite agree with Dr. Bell's view of an existing analogy in the growth we meet in coccidiosis and the tissue changes produced by the Gongylonema. The new-formed coccidiosis growth is strictly a granulation tissue undergoing a degenerative process later, whilst here we have an epithelial growth developing into typical cancerous tissue in its final development. Nor does the parasite of coccidiosis in its adult form attain a liberated free motility; it remains encysted.

3. Appositional Growth in Crown-Gall Tumors and in Cancers

Dr. Erwin Smith (Washington):

SUMMARY

Numerous lantern slides were shown indicating clearly that the growth of crown-gall is chiefly peripheral, as in cancer, and that the lobes of the tumor enlarge by the conversion of a narrow ring of normal tissue into tumor tissue. The full paper has been published in this JOURNAL.

In closing, Dr. Smith called attention to the fact that there is no agreement among oncologists as to appositional growth in cancer. Virchow and practically all oncologists down to Ribbert maintained that primary tumors grow by conversion of surrounding tissues of the same type into tumor cells, i.e., by apposition. Ribbert and his school, on the contrary, denied this *in toto*. Leaving Virchow and his colleagues out of consideration because their work is now old, modern workers as well qualified to judge as Ribbert, Borst, and Borrmann, i.e., such men as Hauser, Hansemann, Ziegler, Menetrier, and others, have strenuously maintained that growth by apposition does occur in primary cancers and some of them have offered instructive figures in proof of their contention. The speaker concluded by saying that the question must be regarded as still unsettled. If growth by apposition does occur in cancer, then, in the light of what he has just shown, it points strongly toward the parasitic origin of cancer. If, on the contrary, it does not occur, parasitism is probably not the cause of the disease.

DISCUSSION

Dr. Isaac Levin (New York): About sixty-five years ago, Virchow, in his book, began the study of cellular pathology with plant cells. He then compared animal with vegetable tumors, and urged pathologists to become acquainted with botanical material and to compare plant and animal tumors, and other pathological conditions. However, we pathologists have forgotten this advice, and when I began a few years ago to be interested in the study of plant neoplasia it was not Virchow's opinion, but a perusal and study of the splendid work of Dr. Smith, that made me take up the problem. In the course of six years' work I have occasionally disagreed with one detail or another of Dr. Smith's findings, but the life of a scientist would be very monotonous if he had no arguments with his fellow-scientists. I am sure that Dr. Smith has always taken my arguments in the right spirit and as a stimulant to further research. But outside of that, I wish to express my appreciation, and I am sure that I voice the sentiment of every man in this country or elsewhere who is doing cancer research, when I express our realization of the splendid contributions Dr. Smith has made to cancer research, and I wish to congratulate him here on his years of very valuable labor.

Dr. Michael Levine (New York): I should like to ask how frequently do nuclear divisions occur in the crown-gall. I have been comparing them with epitheliomas, and I believe that mitoses are not so common as they are there. Perhaps in Washington crown galls can be grown under better conditions than we have in New York.

Dr. Smith: In reply to Dr. Levine's question, I would say that there are just as many mitoses in crown-gall as there are in cancer, but to

see them the material must be collected and fixed in the middle of the night, the best period being from midnight to about four o'clock in the morning. The tumors which furnished the sections I have shown today were collected in the early afternoon and there is less than one mitosis per field of the microscope. Had they been collected in the middle of the night there would have been a number of mitoses in each field. There are some amitotic divisions of the nucleus in crown-gall, but the bulk of the divisions is by mitosis, the same as in cancer. I have found cells in crown-gall with two nuclei, and more rarely with four, and once as many as seven nuclear fragments, but in general these multinucleated cells are exceptional, and in these particular tobacco tumors they occurred almost entirely in the transition tissue on the margin of the tumors.

4. ATTEMPTS AT THE PRODUCTION OF CANCER BY RADIUM

Dr. W. S. Lazarus-Barlow (London):

SUMMARY

This paper, illustrated by lantern slides and microscopical sections, described attempts at the production of carcinoma in animals by means of radium. Starting from his researches (1) on a stimulative action upon the division of ova of Ascaris megalocephala and upon muscular activity (frog) exerted by the rays of radium; (2) on the presence of radium in cancerous tissue; and (3) on the presence of radium in certain gallstones, the speaker discussed the appearances produced by the introduction of radium beneath the skin of rats and of radium-containing gallstones into the gall bladders of rabbits. He submitted that the changes produced are histologically indistinguishable from early carcinoma, being of the squamous cell type in the rat's skin and of the columnar cell type in the rabbits' gall bladder. In the case of one rabbit a true metastasis occurred. In the case of rats the fact that removal of the radium (whether through ulceration or by operation) was followed by retrogression of the early appearances suggestive of carcinoma, led him to endeavor to break down artificially the natural immunity against cancer which rats seem to possess. This was done by (a) feeding with potassium metaphosphite; (b) by repeated, moderately severe irradiation with x-rays over a period of eight to ten weeks; or (c) by a combination of the two agents. When radium was introduced intraperitoneally into rats thus prepared, a larger percentage of cases presenting histological criteria suggestive of malignant disease occurred. The author regarded the experiments as still inconclusive, in the sense that no rat presented a clinical picture recalling malignant disease in man. The lesions were always small in the immediate neighborhood of the radium tube, however closely they might resemble carcinomatous material histologically. He felt that if only it were possible to break down this natural immunity in the rat and produce a

definite carcinoma at will, a great step would have been taken in our knowledge of the disease. Experiments are still proceeding on similar lines at the Middlesex Hospital, and in addition tar is being used as the exciting agent.

The speaker also referred briefly to a projection apparatus, capable of being used in diffuse daylight and operated by the lecturer himself in class demonstrations. The essential points are, that a gas-filled incandescent lamp of 1500 c.p. replaces the arc, and that the image is thrown upon a ground glass screen and viewed by the students by transmission.

5. PRIMARY SPONTANEOUS TUMORS IN THE KIDNEY AND ADRENAL OF MICE: STUDIES ON THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE. SEVENTEENTH REPORT

Miss Maud Slye, Miss Harriet F. Holmes, and *Dr. H. Gideon Wells* (Chicago): Presented by Dr. H. Gideon Wells:

SUMMARY

Primary tumors of the kidney occur not infrequently throughout the animal kingdom, and, in general, seem to exhibit the same variations and peculiarities seen in human renal tumors.

The authors reviewed the literature on the occurrence of renal tumors in animals. All told, they can find records of but 6 cases hitherto reported in mice, all being of epithelial character. In rats, both wild and tame, renal tumors are somewhat more frequent; of 156 reported cases of rat tumors, 27 were in the kidney, all epithelial despite the fact that the other tumors of rats are often sarcomatous. Swine seem to be the only domestic animals in which renal tumors are common, the Wilms type of mixed tumor being most often seen. Renal tumors are not rare in horses, but they are very infrequent in cattle or sheep. No reports of adrenal tumors in mice have been found.

In a series of 33,000 autopsies on mice of the Slye stock, dying natural deaths at all ages, but as far as possible living out their natural span of life, there have been observed the following cases of true primary neoplasm arising from renal or adrenal tissues: (1) From the kidney, 16 tumors, classified as follows: 1 carcinoma, 3 adenomas 1 hypernephroma, 7 sarcomas, 3 mesotheliomas, and 1 sarcoma of the renal pelvis. (2) From the adrenal, 4 tumors, as follows: 1 cortical adenoma from a misplaced inter-renal adrenal rest, 3 mesothelial tumors. (3) Five cases of tumors of the mesothelial structure characteristic of urogenital anlage neoplasms, but the exact origin of which could not be determined because of their widespread growth at the time of death. As these 25 tumors occurred in 33,000 mice presenting not far from 5000 other tumors, they are evidently uncommon tumors of mice, at least in this particular stock.

It will be noted that in this series there has been no instance of a mixed renal tumor of the Wilms type, which is so common a type of renal tumor in man and apparently also in swine. Although inflammatory conditions are very prevalent in the kidneys of mice, epithelial tumors are rare, and especially to be noted is the absence of even a single case of typical malignant hypernephroma, although one benign growth of this type was found. Also no epithelial tumors of the renal pelvis were found, although there was 1 case of sarcoma that seemed to take its origin in the pelvis.

Several instances of malignant retroperitoneal tumors have been observed, mostly of sarcomatous structure, which usually invade the kidney. These have not been included in this series, except 2 cases in which the structure resembled that of the mesotheliomas, suggesting that the tumor had its origin in misplaced rests of the urogenital anlage.

Secondary tumors have never been found in the adrenals, and but rarely in the kidneys. Although this series includes at least 3000 cases of mammary gland carcinoma, often with widespread metastases in the lungs, we have never seen a secondary growth in the kidney. The only secondary carcinomas of the kidney as yet observed are four cases in which the primary carcinoma was in the lung, thus establishing the true neoplastic nature of these lung growths. In but 2 cases have hematogenous metastatic sarcomas been seen in the kidney, if we exclude the numerous cases of invasion of the kidney by direct extension from pararenal growths.

As to sex, taking the entire group of renal and adrenal tumors, there was an equal number in males and in females, agreeing with the observation made with other tumors in mice that, in tumors not peculiar to the sex glands there is usually little difference in incidence in the two sexes.

Differing from the tumors previously studied, coincidence of other tumors with the renal and adrenal tumors is uncommon. One mouse had a spindle cell sarcoma of the thigh. One had a small, benign papillary adenoma of the lung. Only 2 mice had a mammary gland carcinoma, and one of these (21663) was a remarkable case, for this animal, although but one month and eighteen days old, had two independent mammary gland carcinomas, osteosarcomas in the spinal column and in a rib, and a mesotheliomatous type of growth involving both kidneys. Except for this unique case there have been practically no instances of malignant tumors in mice less than four months of age, and few under six months. Most of the renal sarcomas occurred between the ages of seven months and one year, which is somewhat earlier than the usual time of appearance of epithelial growths; this, of course, corresponds to experience with human neoplasms.

The epithelial renal-adrenal tumors furnished no illustration of metastasis, but in 3 cases of sarcomatous or mesotheliomatous growths there was noted involvement of the adjacent lymph nodes, in 2 there were pulmonary, in 2 hepatic, and in 1 splenic metastasis, and in 1 case there were numerous peritoneal growths. The mesothelial type of growth

produced the most extensive metastasis and the most widespread infiltration of the body wall.

DISCUSSION

Dr. Bell: I once studied a series of 100 malignant tumors of the adult kidney. They are very different from those of the child. In that series there were 2 fibrosarcomas in which collagenous fibrils could be identified, so that there was no doubt of their being sarcomas. All the others ranged from a definite adenocarcinoma down to a round cell sarcoma, and every possible transition between carcinoma and sarcoma was observed. In some of the tumors there were definite tubules in the primary renal tumor, and the metastases had the appearance of a round cell sarcoma. These variations are clear if one keeps in mind the origin of the kidney from the metanephrogenous tissue. For that reason it seems to me that we should not make any distinction between these different types of tumors. The objection to the term mesothelioma is that it is a comprehensive term for all of the tumors of the ovaries, testes, and kidneys, and cannot be differentiated from the sarcoma or carcinoma by structural peculiarities. I should like to ask Dr. Wells if he has any objections to the term nephroma.

Dr. Levin: If one could have an experimental laboratory with 10,000 human beings and keep on inoculating the tumors from one to another, I am sure that human tumors would also be found inoculable.

Dr. Wells: I prefer the term "mesothelioma" for the very reason Dr. Bell disapproves of it. It is a general term, and in many of our cases I cannot tell whether the mass arises in the adrenal or in the kidney, but it evidently arises from the mesoblastic embryonal tissues and so, therefore, I use a general term. I do not like the tendency to adopt the term "nephroma," any more than I like "thymoma," and similar terms. We would have equally to use "hepatoma," "cerebroma," and so on, and it would make an absurd nomenclature. The term mesothelioma does mean tumors arising from a mesoblastic origin and producing epithelial structures, showing every possible transition between one form and another.

6. On the Rôle Played by Carbon Dioxide in Controlling Cell Proliferation

Dr. G. H. A. Clowes and Homer W. Smith (Indianapolis).

7. The Action of Radium Emanation on Neoplasias in Plants

Drs. Isaac Levin and Michael Levine (New York):

In previous communications the writers have shown that plant tumors present an ideal material for the study of the direct action of *x*-rays and radium on the tumor cell. The so-called "buried emana-

tion" method of radium therapy is constantly becoming of greater importance in the treatment of cancer in the human patient. It consists in placing a minute glass capillary filled with radium emanation into the tumor. Both the softest beta as well as the hardest gamma radiations act on the tumor tissue surrounding the capillary, while the alpha rays are absorbed by the glass.

The present investigation consists in the application of this method to neoplasias in plants in order to ascertain the mechanism of the direct action of radium on the tumor cell. Young growing and adult tissues were used for purposes of comparison, while crown-gall and club root tissues were the main material. Capillary tubules 3 mm. long and 0.25 mm. in diameter, containing from 0.5 to 3 mc. of radium emanation were inserted into growing points of tobacco, adult roots of the turnip, crown-gall tissue of geraniums, and young club root tissue of the turnip. Empty tubules equal in size to those containing the emanations were inserted in identical tissues as controls. The insertion of the radium emanation was done at various intervals after the beginning of the formation of the crown-gall or club root. The development of the radiated tumors was noted and the radiated neoplastic tissues were removed for microscopical examination from one day to several weeks after the insertion of the capillary.

The analysis of the results of these studies shows the following: the insertion of the radium emanation is followed by a general inhibition of the development of the crown-gall or club root, which tallies well with the results obtained by the writers previously by x-raying the crown-gall. Consequently the hard gamma ray fraction of the radium emanation capillary tubes produces the same effect on the tumor as the x-rays or the filtered gamma rays of radium applied at a distance from the tumor.

In the tissues in the immediate vicinity of the tubes deeper changes in the tumor cells were noted. Section of this region shows the collapse of cell walls radially to the needle, forming a cushion of cellulose. The cells immediately behind this cushion are devoid of cytoplasm. Occasionally one finds a nucleus in process of disintegration. In cells further back of this area one finds unchanged nuclei.

In club root tissue the degenerated cells immediately adjoining the so-called cellulose cushion do not seem to contain the Plasmodiophora brassica, while the parasite is present in the cells at a further distance from the capillary. This apparent action of radium on the parasite as well as the more minute study of the intracellular changes caused by the irradiation is a subject of further study by the writers and will be reported later. It is rather significant that the cellulose membrane of the plant cell seems to play a similar rôle in plants in walling off the necrotic area about the emanation tubes and filtering off the soft radiations as the lymphoid tissue stroma does in animal tumors.

8. Defensive Factors Against Cancer

Dr. William Carpenter MacCarty (Rochester):

SUMMARY

Dr. MacCarty called attention to the fact that in the study of post-operative histories and results, one is often surprised to find the patient living nine or ten years after extirpation, though complete involvement of the lymph-nodes had been found at operation. This is especially striking when one compares these cases with others having small cancers and no glandular involvement, who have died six months or two years after operation. The present study was undertaken to determine, if possible, whether there were any factors which might have some influence on this increased longevity. In connection with this the following conditions are considered: lymphocytic infiltration, fibrosis, hyalinization, and the degree of differentiation of the cells of the cancer itself.

The records of 99 gastric cancers, 92 breast cancers, and 102 cancers of the rectum were studied. All the operations were done in the same way by a group of surgeons who had received the same training. All the patients died of recurrent or metastatic cancer.

A study of the relative frequency of the factors just mentioned shows that differentiation occurs in 65 per cent of the stomach cases, 8.6 per cent of the breast cases, and in 86 per cent of the rectal cases. There is, therefore, a great variation in the frequency of occurrence of these different factors in different organs of the body. Fibrosis in the stomach is a rather rare condition. The same is true of hyalinization, but in the breast and in the rectum this factor occurs quite frequently.

Tables were presented to show the combination of the different factors, and the variation in the different organs. A comparison was made of the average length of post-operative life when the factors were present and when they were absent. For example, in the breast with differentiation the average length of life is 3.65 years; without differentiation 2.35 years. Whenever the factors are present, the average length of life is increased over when the factors are absent.

As a result of this study, it appears that there is a constant increased longevity when these factors are present, either alone, or in the various combinations, and that the greatest increase is associated with differentiation and hyalinization, in combination or alone. Since these figures were collected, a study of epithelioma of the lip and of the skin and other portions of the body has shown the same results. The explanation is left open, but it is known that the more differentiated a cell, the less power of reproduction it has, except the highly specialized sex cells, and also that hyaline tissue is a very dense tissue and that penetrability is in proportion to the density.

9. Preliminary Report on Tumor Growth Following Injection of Radiated Tumor Emulsion

Dr. M. J. Sittenfield (New York).

10. BIOLOGICAL EVIDENCE FOR THE INHERITABILITY OF CANCER IN MAN

Miss Maud Slye (Chicago):

ABSTRACT

The biologic problem of the nature and inheritability of spontaneous cancer has been under study in this laboratory for the past twelve years, during which time the results consistently obtained have demonstrated the fact that cancer is inheritable. Among the many thousand mice bred in this laboratory in the study of cancer heredity, there has been no case that does not agree with this conclusion—no appearance of spontaneous cancer in a non-cancer strain, nor any line into which cancer has been bred where it has not appeared in exact accordance with the known laws of heredity. The inheritance behavior of cancer has consistently been that of a simple Mendelian recessive.

It is therefore a demonstrated fact that cancer is inheritable in mice, and this fact is now pretty generally accepted.

The profound and biologic aspect of this demonstration of the inheritability of cancer, however, has for the most part failed to be grasped by the pathological and medical world, and the object of this paper, therefore, is to emphasize that phase of the study, and to make clear the exact application of this demonstration to the problem of the ininheritability of cancer in man.

Every instance of organic behavior is based upon biological law. Many of these laws are as yet not even foreshadowed. In the case of the law of heredity, however, which is the most fundamental and most potent of all biologic laws, we have the fundamental facts. But although we have these facts, we continue in actual opinion and practice for the most part entirely to ignore them.

If we give heredity its full biologic definition, we must define it as follows: Heredity is the force which makes and holds together the genus and the species. It determines what form, what characteristics and what activities, in the most complete sense of the words, every genus, every species, and every individual shall have. It determines the beginning of every organism as a single cell, each (after the unicellular forms) in its development undergoing cell division and differentiation, and each recapitulating in hurried fashion the history of organic evolution which has preceded it. This is true of man as it is of every other organism which antedated him in the process of evolution. Each organism has been made from organisms which preceded it, and which set aside the germ plasm, with all its potentialities, from which the offspring develops.

Out of this fact grows the biologic law of heredity which underlies all life: That which goes into the germ plasm must come out in the offspring. This applies to every living organism from unicellular plants to man. Each individual develops out of germ plasm laid down with all its potentialities by individuals which preceded him; in every item made out of material furnished by his ancestors.

Man, then, developing from germ plasm laid down by his ancestors, inherits the same type and behavior of tissues shown by his ancestors.

The method of heredity was worked out by Mendel with green peas. Later and following him Cuenot and others worked it out with mice, and found the method of heredity identical in mice with that in peas.

In the scheme of evolution mice are far removed from peas, but closely related to man, who also belongs to the mammalian group. The structures of mice are like those of man, their tissues behave like those of man because they were derived from a common ancestry. This is the functioning of the law of heredity which transmits each type of protoplasmic behavior down the full line of evolution.

This is the heart of the theory of evolution and without it nothing remains of that theory, which is no longer questionable, but a working tool in science from astronomy to psychology.

What goes into the germ plasm comes out in the offspring. Similar tissues must behave in similar fashion if there is to be such a thing as species or race. Without this fundamental fact the organic world would be chaos. Similar tissues derived from a common ancestry must behave in a similar way.

The mouse tumors under study in this laboratory are spontaneous neoplasms arising in the natural life of the animal without artificial interference of any sort except that of selective breeding, exactly as man's spontaneous tumors arise. They arise in the same tissues and in the same organs as the tumors of man; they follow the same clinical course; they cause death in the same ways. Under the microscope they present the same appearance as similar tumors in similar organs in man. *They are the same biologic entity as similar tumors in man. And consequently if we do not discard the entire theory of evolution, we must admit that they behave in the same way in the matter of heredity as in all other matters.*

Moreover, accurate human statistical evidence when it is correctly and biologically read, also demonstrates the inheritability of cancer in man.

As already stated, cancer behaves like a Mendelian recessive, like albinism and spotting and the whirling habit of the Japanese waltzer. If only recessives are bred, the dominant will be lost. If this type of breeding were exclusively pursued, the non-cancer tendency would be lost, just as the pigment-making tendency would be lost if only albinos were bred. Recessives cannot transmit the dominant.

The suggestion is also made that in order to avoid artifacts in our animal experimentation, biologically analyzed stocks must be used.

RECAPITULATION

1. Cancer and non-cancer segregate out and are transmitted as such.
2. They are therefore unit characters.
3. A specificity of tissue type from organ to organ segregates out and is transmitted as such.

4. It is therefore a unit character.

5. Since these things are unit characters, it is possible to manipulate them by selective breeding and thereby to implant them indelibly in any species, or to *eliminate them permanently and completely from any species*.

6. Cancer and non-cancer behave like the absence and presence, respectively, of a mechanism fitted to control proliferation and differentiation in regenerative processes, and an animal either has it, or lacks it, no matter to what species he may belong.

7. There is therefore a ready and certain genetic method of escape from cancer for the individual and the race.

8. The demonstration of the inheritability of cancer in mice is a demonstration of the inheritability of cancer in man and in all other species which show it, if we are to maintain the theory of evolution.

9. The study of cancer behavior, which has demonstrated itself to be fundamentally a biologic problem, points the way to the understanding of all pathologic conditions.

10. And, therefore, when biology underlies all our pathology and bacteriology, all our physiology and therapy, there will no longer be these monstrous diseases, but only the slow and natural death, which is the fatigue and diminution and final cessation of the organ and the organism.

OF GENERAL BIOLOGIC IMPORT

1. From the procedure of analyzing stock into its unit characters in order to learn how to manipulate cancer, there has emerged the fundamental law of heredity—what goes into the germ plasm comes out in the offspring.

2. Every organism is a complex of unit characters and cannot be correctly interpreted or manipulated in experiments with a certain outcome, until it has been analyzed into its unit characters.

3. The unit character as the biologic ultimate, like the ion in chemistry, is the explanation of all biologic and hence of all pathologic syntheses. And all such syntheses can be understood and manipulated, only when they have been analyzed into unit characters.

DISCUSSION

Dr. Smith: I have listened to Miss Slye's paper with the greatest interest, and I am willing to concede in the matter of heredity that one may reason from mice to men. I believe she has established clearly that if we could select our ancestors we could all escape cancer. In other words, if we could breed men as we breed mice, a race of men could be developed immune to cancer. But there are two factors constantly operative on all living things, only one of which has been stressed by Miss Slye. These two factors are heredity and environment. She has shown clearly that by breeding mice from cancerous parents she has obtained a race 100 per cent cancerous, and by elimina-

tive breeding a race 100 per cent free. I believe the same thing could be done with crown-gall or with tuberculosis; and yet that would not prove that the two diseases I have mentioned are due to heredity. So I believe that in all probability there is a cancer parasite able to act on certain strains of her mice, and not able to act on others. If it occurs at all, it is probably some organism transmitted from cage to cage and mouse to mouse by some common ectoparasite. I can think of whole families of persons who died of tuberculosis—father, mother, and half a dozen children—and there the tendency was very strong, but the tendency itself was not the cause of the disease.

In the case of crown-gall in very susceptible species, I would not stress the necessity of pure strains as Miss Slye is inclined to do; for, taking the plants as they run, in these species I can get 100 per cent of takes in my inoculations. I have now about 50 inoculated Pelargoniums, every one of which has taken the disease, but in other plants, like the rose, there is great variation in susceptibility.

Dr. Sturges: May I ask whether, in the case of chimneysweeps' cancer, where environment plays such a very large part, there is a question of heredity underlying the cancer? And does this apply to the experimental cancers of rabbits' ears?

Dr. Rosenstirn: If the laws of cancer heredity in mice are equally valid for man, then, in the human being, where both parents have cancer, would you expect an unbroken series of cancer in their direct offspring, that is, in those who lived long enough to develop the disease?

Miss Slye: I must object to Dr. Smith's using as an argument the assumption (which must remain merely an assumption until it has been demonstrated experimentally) that you can breed out either tuberculosis or artificially induced crown-gall. In the meantime the fact that spontaneous cancer is hereditary is experimentally demonstrated.

I have not in this paper made any mention of the parasite theory of the origin of cancer, nor is there occasion or time to do so in the discussion. Moreover it is the strong, *and not the weak* individuals that develop cancer both in mice and in the human species. Herein cancer is conspicuously different from known infections, as it is conspicuously different from them in many other ways.

In reply to Dr. Sturges: The stocks both of rabbits and mice which have been used in the production of "coal tar cancers" were not in any case tested as to their tendency to spontaneous cancer. It is therefore impossible to say whether or not they would have produced spontaneous tumors. The chimneysweep and x-ray cancers of man seem to me to be closely related to these experimentally produced cancers in animals. This subject will be discussed in another paper, which I was unable to prepare for this meeting.

In reply to Dr. Rosenstirn: In the human species where both parents have spontaneous cancer, I would certainly expect cancer in the

offspring who lived to cancer age, and who were subjected to the type of chronic irritation fitted to occasion cancer of the type and in the organ predisposed by heredity to such cancer. The paper which I have just presented was intended to answer exactly the question which Dr. Rosenstirn has asked.

A TRANSPLANTABLE METASTASIZING CHONDRO-RHABDO-MYO-SARCOMA OF THE RAT

F. D. BULLOCK AND M. R. CURTIS

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With the exception of certain mixed tumors of the testis, ovary, and kidney, neoplasms containing striated muscle are very infrequent in man, and only a few have been described in animals. In a comprehensive review of the literature on animal tumors, in 1896, Caspar (1) mentioned but 3 cases of rhabdo-myomata, i.e., a melanotic rhabdo-myoma of the tail and perianal connective tissue of a stallion, reported by Kolessnikoff; a rhabdo-myoma of the vagi of a horse, described by Gratia; and an adeno-sarco-rhabdo-myoma of the kidney of a hog found by Johne. J. Wolff (2) cited a case of rhabdo-myoma of the shoulder of a horse, discovered by Monod, and Magnusson (3), quoting Boncek, reported an adeno-rhabdo-myoma of the heart of a cow. A giant-cell rhabdo-myo-sarcoma of a trout was described by Adami (4), and Fibiger (5) has reported a rhabdo-myoma of the codfish. However, the rat tumor described in the present paper is apparently the first rhabdo-myoma to be recorded in a rodent, in spite of the number and variety of neoplasms which have been observed in rats and mice. This tumor is interesting not only for its rarity, but also because of its morphology and its biological character.
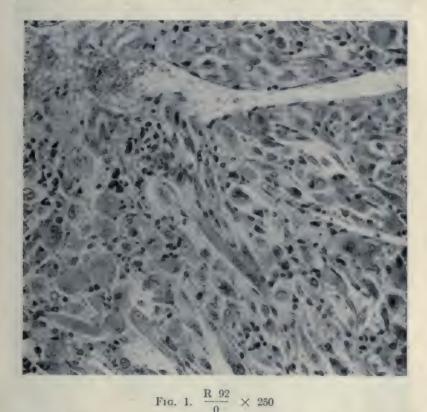
The growth was found in a black and white female rat, between fifteen and nineteen months old, of a group of animals experimentally infested with Cysticercus fasciolaris. This rat, however, had not developed sarcoma of the liver. When first observed the tumor was a small circumscribed hemispherical mass about the size of a pea, situated beneath the skin of the upper epigastrium, in the median line of the body. It was firm and elastic

and was apparently adherent to the ensiform process of the sternum.   During the first few weeks of observation there was no material increase in size of the tumor, but at the end of two months, when the animal was mated, it was noted that the mass had grown slightly.   Three weeks later the rat was isolated pregnant, and it was then recorded that the tumor had about doubled in size since the last observation.   The rat gave birth to two young, only one of which she reared.   Throughout lactation and subsequently the tumor grew rapidly.   One month after gestation, a fragment of tumor was surgically removed, and was used for the subcutaneous inoculation of 93 rats.   At this time the growth measured 5.5 x 3.5 x 3 cm.   The rat survived the operation thirty days, during which period the tumor grew with great rapidity attaining a size of 6.5 x 5.9 x 4.4 cm.   At autopsy the growth surrounded and largely replaced the ensiform process, its capsule being closely adherent to the sternal end of the two caudal pair of ribs.   It was irregularly nodular, distinctly lobulated, and covered by a thin fibrous capsule.   The consistency was firm and tough, with small soft and cartilaginous areas scattered throughout.   In certain parts the tumor was friable and short plugs of tissue could be expressed from the cut surface by pressure.   The color was grey white to pinkish, with small scattered areas of hemorrhage and large and small patches of necrosis.   Gross metastatic tumor deposits were present in the lungs.

Microscopically, the tumor showed a rather complex structure which varied in different parts.   It was composed partly of muscle cells and fibers and partly of small round or polyhedral cells, suggesting embryonal cartilage cells.   These two types of cells occurred separately in parts of the tumor, though generally they were freely intermixed, one or the other predominating.

Figures 1 and 2 respectively show low and high power views of areas of the tumor in which the muscle elements predominate. The muscle elements showed a wide diversity in shape and size many of them being morphologically fibers while others had the appearance of giant cells.   Some of the fibers were thick, short or elongated cylinders with blunt, serrated, or tapering ends;

others were long, slender, and often fusiform, while still others were club-shaped and occasionally branching. They were generally multinuclear and possessed an abundant acidophilic protoplasm. In many of them longitudinal striations were visible and some showed both longitudinal and cross striations

Fig. 1. $\frac{R\ 92}{0}$ × 250

An area of the spontaneous chondro-rhabdo-myo-sarcoma of the rat composed largely of muscle elements.

(see fig. 2); others bore a close resemblance to smooth muscle cells. The giant cells consisted of round, oval, or irregular shaped masses of acidophilic protoplasm containing one to many nuclei, multinucleated cells being the rule. The nuclei of the fibers were generally rod-shaped or oval, while the cell nuclei were mostly

vesicular. They occupied either the central or peripheral part
of the cell, being sometimes arranged in the form of a ring. The

FIG. 2. $\frac{R\ 92}{0}$ $\times$ 1000

A striated muscle fiber from the spontaneous tumor

chromatin was not abundant and was distributed in small
scattered granules. One to two nucleoli were generally demon-
strable. There was considerable cellular degeneration, associated

with irregularity in the shape of the nuclei and changes in their staining properties. In places the degeneration involved the stroma and even resulted in necrosis which often covered large areas. Glycogen was demonstrated in the protoplasm of some of the cells. The fibers were either scattered indiscriminately among the cellular elements or ran in bundles. These bundles were most abundant at the periphery of the tumor and at the borders of the lobules. The muscle elements were loosely embedded in a stroma of rather delicate fibrous tissue of moderate cellularity, which in places was arranged in the form of a network, in the meshes of which lay the muscle cells. It either surrounded individual cells or divided the cells into small groups. The cells did not completely fill the spaces, but lay free in the meshes of the network. This appearance was probably due to shrinkage incident upon fixation and embedding of the tissues.

The other cells which formed a constituent part of the tumor were mostly rather small and round or polyhedral in shape. They possessed relatively large vesicular nuclei and scanty cytoplasm. The chromatin of the cells varied in amount and was distributed in small granules. The nucleolus was represented by one or two large granules. These cells for the most part showed but little difference in size, and generally took a uniform stain; though in areas they were fairly large and hyperchromatic. Most of them possessed a single nucleus, though cells containing two to several nuclei were sometimes observed. They were usually more compactly arranged than the muscle elements and were occasionally split up into islands by strands of stroma. In areas they formed a loose meshwork which enclosed the muscle elements. Mitotic figures were present in both types of cells and were particularly abundant in the small cells. The stroma throughout the tumor was relatively scanty, although in places bands of fibrous tissues penetrated the growth dividing it into distinct lobules.

Scattered through the tumor were large and small islands of cartilage (see fig. 3). The central parts of some of these islands consist of differentiated cartilage cells, while the cells comprising other islands were solely embryonal in type. The cartilaginous

islands showed a marked tendency toward necrosis.   Embedded
in the tumor were several small trabeculae of osteoid tissue.   The
tumor was rich in blood-vessels consisting largely of dilated
capillaries.

The metastatic nodules in the lung contained both types of
cells, the muscle elements preponderating.   In these secondary
tumors the muscle elements were smaller than those of the
primary growth and showed no definite cross striations.
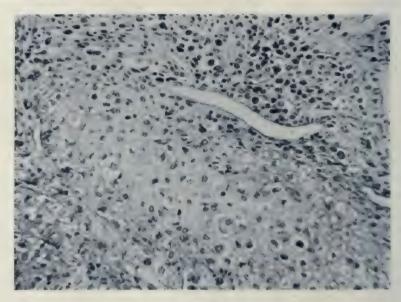
FIG. 3. $\dfrac{R\ 92}{0}$ $\times$ 250

An island of adult cartilage with a part of the surrounding tissue composed of
small round or polyhedral cells resembling embryonal cartilage cells.

Transplantation of the tumor was successful and it is now in
the eighteenth generation.   In its initial difficulty of propagation
this tumor resembled a carcinoma rather than a sarcoma.   Of
the grafts introduced subcutaneously into the 93 rats of the first
generation only three produced tumors, and only five tumors were
obtained from the 48 rats of the second generation.   The third
generation, however, showed 80 per cent of successful inoculations

Fig. 4. $\dfrac{R\ 92}{9\ B}$

A rat bearing a transplanted tumor of the ninth generation photographed 110 days after inoculation. The tumor weighed 2.14 times as much as its host.

and the subsequent generations showed generally a moderate
to a high percentage of takes.   The rate of growth of the trans-

FIG. 5. $\frac{R\ 92}{1\ A}$ $\times$ 160

An area of a tumor of the first series transplanted showing muscle giant cells
and fibers and a few of the round or polyhedral cells.

planted tumors has also increased since the early generations.
The daughter tumors often attain enormous sizes, sometimes
greatly outweighing their hosts before death ensues.

Figure 4 shows a photograph of a young rat bearing a tumor of the ninth generation.   This animal was killed and photographed

Fig. 6. $\dfrac{\text{R } 92}{4 \text{ A}}$ $\times$ 800

Striated muscle fibers in a transplanted tumor of the fourth generation

one hundred and ten days after inoculation. After the removal of the tumor the rat weighed 105 grams while the tumor weighed 225 grams or 2.14 times as much as its host.

Unlike most other rat sarcomata this tumor on transplantation grows progressively in almost every animal in which a graft becomes established. The older transplanted tumors show a tendency toward liquefaction necrosis.

Tumors resulting from each of the first 13 successive inoculations have been examined histologically. They bore a close

FIG. 7. $\dfrac{\text{R } 92}{\text{1 A}} \times 1000$

Multinucleated fiber in a transplanted tumor of the first generation showing a mitotic figure.

resemblance to the spontaneous tumor except that osteoid tissue was absent in all of them and cartilage was observed in only 2, both of which were derived from the first transplantation. All the transplanted tumors examined contained the other constituents in varying proportions, from tumors consisting largely of muscle to others in which the small round or polyhedral cells predominated. The muscle elements consisted of both fibers and cells as in the original tumor.

Figure 5 is an area of a tumor of the first series inoculated showing many muscle giant cells and fibers and a few of the small cells. The continued presence of fibers with cross striation in each successive generation is worthy of note; in fact, cross striations were demonstrated in some of the muscle fibers of every transplanted tumor examined.

FIG. 8. $\dfrac{R\ 92}{15\ A}$ × 400

Lung metastasis of a transplanted tumor of the fifteenth generation showing both muscle elements and the smaller cells.

Figure 6 shows cross striated fibers in a tumor of the fourth generation. That the muscle elements were growing actively was evidenced by the presence of a large number of mitotic figures. These division figures were frequent in the cells and sometimes there was observed in a fiber a karyokinetic figure. Such a nuclear division in a fiber is shown in figure 7.

One tumor obtained from the first transplantation produced lung metastases, which contained a relatively small amount of muscle in which cross striations were not demonstrable, and one tumor from the fifteenth transplantation produced very extensive lung metastases which contained a large amount of muscular tissue.

Figure 8 shows an area from one of these lung metastases in which both types of cells are represented.

Fig. 9. $\dfrac{R\ 92}{3\ A}\ \times\ 500$

An embolus in a blood-vessel of a transplanted tumor of the third generation

Emboli were occasionally observed in the blood-vessels of tumors of later generations (see fig. 9).

SUMMARY

The chondro-rhabdo-myo-sarcoma of the sternum of the rat described above was a transplantable metastasizing tumor in which cross-striated muscle fibers have persisted through fifteen generations, although the cartilaginous elements early lost their power of differentiation.

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PRIMARY CARCINOMA OF THE LIVER

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INTRODUCTION

Primary carcinoma of the liver occurs in 0.028 to 0.03 per cent of all necropsies according to the statistics collected by Winternitz. A number of cases have been reported in the literature but this form of cancer occurs very rarely in the experience of any one pathologist.

It is the purpose of the present investigation to compare two cases of carcinoma of the liver, the first case being that of apparently primary growth in the liver while the second case had its primary focus in the gall bladder, from which the growth directly invaded the liver. These cases occurred at successive necropsies.

The following questions concerning the case of primary carcinoma will be considered: (1) whether the cancer is derived from liver cells or from cells of the smaller bile ducts; (2) whether there is any relation of the malignant growth to cirrhosis; (3) whether the growth takes place by a gradual metamorphosis of liver cells into cancer cells, or by autocellular proliferation; and (4) whether the cancer is multicentric or unicentric in origin.

According to Wegelin the most important and reliable differential feature between cancers derived from liver cells and those derived from the cells of the small bile ducts is the nature of the stroma, liver cell cancers having a capillary stroma, while the bile-duct-cell cancers have a predominantly connective tissue stroma.

The majority of investigators believe that cirrhosis precedes primary malignancy and is the causative factor. Cirrhosis is thought to be preceded by injury and followed by hyperplasia

of the liver cells. The hyperplasia continues and takes on malignant character.

v. Heukelom and other investigators have found in cases of primary carcinoma of the liver what appeared to be transitions from the liver cells to cancer cells and have interpreted such findings as appositional growth in which a gradual metamorphosis from liver cell to cancer cell took place. This hypothesis has been attacked chiefly by Ribbert, who claims that the growth of primary liver cancer is from one focus.

Because of the demonstration of the so-called transitions in multiple small cancerous nodules in the liver, v. Heukelom, Travis, and others considered that primary liver cancer was multicentric in origin. Ribbert and Winternitz, on the other hand, deny the multicentricity of origin, and their investigations tend to show that the neoplasm arises from a primary focus and that the growth develops by extension and metastases through the portal system.

I find in the cases here to be reported that there is a close similarity between some of the microscopic pictures presented by the primary cancer and those presented by the cancer arising in the gall bladder and secondarily invading the liver. In the case of primary carcinoma the neoplasm apparently arises from the liver cells. Cirrhosis is not marked and is secondary to the neoplasm. The mode of growth is autogenous, and the origin is unicentric.

REPORT OF CASES

1. A case of primary carcinoma of the liver.

Clinical Note. The patient, a white woman sixty-three years old, was admitted to the University of Virginia Hospital, March 26, 1920, complaining of weakness, nausea, and rather vague pains in the abdomen. The patient began to lose strength and weight about two months prior to admission. About three weeks before admission her abdomen became distended and a little later she became jaundiced. On physical examination the margin of the liver could be palpated below the costal margin. A diagnosis of carcinoma of the liver was made. Three days after admission the patient died.

Necropsy note. The body is that of a rather well nourished old woman, with an intense yellow color. The abdomen is prominent and rather tense.

Upon opening the peritoneal cavity a large amount of bile stained fluid escapes. The liver extends about 5 cm. below the costal margins. The omentum is adherent to the liver and gall bladder by easily separated adhesions. There are enlarged and very hard nodules in the gastrohepatic omentum. The lymph nodes of the mesentery and small intestine are not enlarged. The intestines show subserous ecchymoses.

Upon opening the pleural cavity there is no free fluid found. There are a few old fibrous adhesions between the lungs and the chest wall. The pericardial cavity shows a normal amount of bile stained fluid. The lungs show a recent process of bronchopneumonia in the right upper lobe. The heart, spleen, stomach, intestines, pancreas, adrenals, ureters, bladder, uterus, ovaries, and the thyroid show nothing of special interest and no evidence of neoplastic change. The kidneys present the picture of chronic interstitial nephritis. The retroperitoneal tissue presents no evidence of malignancy. The wall of the gall bladder is thickened and upon opening it there is found a dark almost tarry bile, and several brown rather hard stones. There is no tumorous growth of the gall bladder. The cystic and common duct traced into the intestine show no obstruction. The brain and spinal cord are not examined.

Microscopic study of the above-mentioned tissues fails to demonstrate any malignant neoplasm. There are metastases to the lymph nodes of the gastrohepatic omentum.

The liver is slightly enlarged and presents a very peculiar appearance. There is a background of grass-green tissue with yellow tracings. Splotched about on the surface are areas, roughly circular in shape, of a grayish yellow color with a depressed center and a slightly raised border, and with a suggestion of radial striations. In some cases these areas have a pinkish tinge due to congestion. The average size of these areas is about 2 cm., and they cover about half the surface area of the liver. The liver borders are sharp. The whole liver is rather firm and the above-mentioned spots seem firmer than the surrounding tissue.

On gross section, again there is discernible a grass-green background traced with yellow and punctuated with the above-

described roughly circular spots which appear more granular than the liver tissue (fig. 1). The centers of these spots retract promptly after section. The greater portion of the neoplasm is situated in the right lobe and here was probably the primary focus. The veins do not seem to be involved in the cancerous growth and are patent.

Microscopically a variety of pictures are seen. These may be divided into three general types: (1) fields in which only liver tissue is seen; (2) fields containing both liver tissue and malignant tissue, and (3) fields exclusively cancerous.

Type 1. The liver tissue shows all stages of degeneration. The more normal cells show cloudy swelling, while in the areas of greatest degeneration fatty changes are marked. Degeneration seems to be more marked at the periphery of the lobules. There is no evidence of regeneration or hyperplasia. No mitotic figures are seen, but occasionally cells with double nuclei are present. There is no increase in the stroma. In some areas the capillary sinusoids are congested. The bile ducts appear normal and there are no cancer cells in the portal veins.

Type 2. The picture presented by this type of field gives the impression that the liver tissue is melting away before the invading cancer cells. First, there is a zone of liver cells which are in a fair state of preservation. From this zone there is a gradual transition into a second zone in which degenerative changes of the liver tissue are marked, and become more marked as the zone formed by the cancer cells is approached.

In the first zone, with the exception of cloudy swelling of the liver cells, there is nothing abnormal. In the second zone degeneration becomes marked, and the cytoplasm of the cells is filled with vacuoles (figs. 3 and 4), probably due to fatty changes. The nuclei are small and stain deeply. There is no evidence of regeneration of the liver cells. There is no increase in the stroma and the arrangement of the cells is normal. The cancer cells of the third zone are arranged in trabeculae (figs. 3 and 4). The predominant cell is polyhedral and somewhat larger than the liver cell. The cytoplasm is granular and stains a bright pink with eosin. The nucleus is large and hyperchromatic.

Mitotic figures are numerous in this region and occasionally an atypical figure can be observed (fig. 3). In some cases there is direct continuity between the column of cancer cells and the liver trabecula, but the cancer cell and the liver cell in apposition with it are sharply differentiated (figs. 3 and 4). There is no evidence of a transition from liver to cancer cells; the liver cells next to the cancer cells are necrotic. The stroma is composed of capillaries. The invading cancer cells make use of and grow between the pre-existing parallel capillaries of the liver trabeculae (figs. 3 and 4).

Type 3. Passing from the newer regions of the cancerous growth above-described into the more mature areas, the picture is somewhat altered. The stroma is increased, and while it is still made up largely of capilllaries the connective tissue elements are also multiplied. The cancer cells are divided into islands by the stroma, and the trabecular formation is lost. The cells are somewhat smaller than the newly formed cells, and the nuclei take a deeper stain. There is no mitosis but some of the cells have double nuclei. The central portion of a large cancer nodule often shows necrosis.

2. A case of primary carcinoma of the gall-bladder, with invasion and metastases in the liver.

Clinical note. The patient, a white woman, sixty-three years old, was admitted to the University of Virginia Hospital March 23, 1920, complaining of loss of weight and progressive weakness. Two months prior to admission a mass was noted in the abdomen. The patient died eight days after admission.

As the present interest in this case centers about the liver, the details of the necropsy will be omitted.

The liver is about one and a half times its normal size, and the right lobe shows the most enlargement. The enlargement in the right lobe is accompanied by numerous nodular, irregular protuberances with discolored areas looking like venous blood. These nodular protuberances remind one very much of bunches of varicose veins just beneath the liver surface. In other areas,

notably in the left lobe, there are also enlargements which, however, do not show the blood color but appear as nodular pale white lumps beneath the surface. Between these two extremes are various stages. The liver tissue between these raised areas is pale, mottled, and yellow. The organ is rather soft in consistency, the varicose areas above-mentioned give a semi-fluctuating sensation.

On gross section a confusing array of appearances is seen, and as the liver is cut a quantity of dark bloody grumous material escapes as if under pressure. Very little liver tissue can be seen on section through the right lobe. The varicose nodules appear as dirty, grayish, soft, granular tissue, interspersed with numerous spaces containing dark, semi-fluid blood. These areas vary in size and seem to be well circumscribed. The liver in places shows a great excess of fibrous tissue. There are some areas which appear as dirty whitish growths that do not show much hemorrhage (fig. 2). These correspond to the nodular growths seen from the surface.

The surface of the gall-bladder is roughened, due to the separation of adhesions between it and the transverse colon. The wall of the organ is thickened, and upon being opened a mass of fungoid, pale, rather soft tissue is found nearly obliterating the cavity. The cancerous wall is thick and cartilaginous (fig. 2). In the fundus is a solitary oval stone. The growth in the gall-bladder bears a close resemblance to the cancerous extensions in the liver.

Under the microscope a great portion of the tissue from the liver shows hemorrhage and necrosis. In the less affected areas the pictures differ according to the age of the malignant invasion of that region. In the more mature regions the nodules of cancer cells are surrounded by dense bands of connective tissue. The cancer cells are cuboidal. They have a granular cytoplasm and large hyperchromatic nuclei. Some of the cells contain double nuclei, but mitotic figures in these areas are rare. In some places the cancer cells have invaded the surrounding connective tissue and entered the blood vessels. There is a round cell infiltration of the connective tissue stroma and in places groups

of hyperplastic liver cells are seen. These groups of cells have an irregular arrangement which bears no relation to the normal arrangement of liver cells. The cytoplasm of these cells takes a bluish tint with hematoxylin and eosin staining, and the nuclei are hyperchromatic. These cells are easily distinguished from carcinoma cells (fig. 5).

In the younger regions the picture is one of invasion of the liver tissue by cancer cells, with degeneration and disappearance of the liver tissue before the advancing cancer cells The liver cells proximal to the cancer cells show the greatest amount of vacuolar degeneration. In these areas there is no evidence of hyperplasia of the liver cells. The cells have a normal arrangement and the stroma is not increased. The cancer cells are arranged in trabeculae. The cells are polyhedral, somewhat larger than the liver cells, and contain large hyperchromatic nuclei. Mitotic figures are frequent. In some cases the columns of cancer cells are in direct continuity with the columns of the liver cells and grow by advancing between parallel capillaries of the pre-existing liver tissue, destroying and replacing the liver cells in their advance. Thus, in this region of invasion, the cancer cells have a capillary stroma (fig. 6). In the older regions this capillary stroma is supplemented by the growth of connective tissue which becomes the predominating element. In some instances the cells of this cancer show a tendency to the formation of duct-like structures.

REVIEW OF LITERATURE

The rarity of primary carcinoma of the liver is shown in the following table:

INVESTIGATOR	NECROPSIES	CASES	PER CENT
Goldzieher and v. Bokay (7)	6,000	18	0.03
Wheeler (28)	5,233	15	0.028
Winternitz (30)	3,700	6	0.016
Total	14,933	39	0.024*

* Average per cent.

The condition occurs at all ages, but according to statistics given by Yamagiwa (31) about 50 per cent of cases occur between the ages of forty and sixty years. Statistics by the same author show a slight preponderance in males.

Heredity, as in other malignant conditions, may play a part in primary liver cancer, but in the majority of cases the family history is negative. Hedinger (9) reports primary carcinoma occurring in two sisters. By breeding with selected strains of mice, Maude Slye (23) was able to produce a strain in which the incidence of primary malignancy of the liver was high.

Clinically there is no definite picture produced by the disease. Karsner (13) states that in the majority of cases the symptoms are those of cirrhosis. The earliest symptoms are vague gastro-intestinal disturbances. After the tumor develops there is a loss of flesh, cachexia, and digestive disturbances. Icterus is present in 63 per cent of cases: ascites in 58.5 per cent; edema in 41 per cent; splenic tumor in 32 per cent; and fever in 14 per cent of cases. The condition is not very often diagnosed.

A number of cases have been reported in which primary carcinoma of the liver has been associated with some other disease in the liver. Syphilis has been reported by DeMassary (16). Modena (18) has described cancer associated with echinococcus cysts. In a case reported by Cleland (3) leprosy bacilli were demonstrated.

Primary carcinoma of the liver is a rapidly fatal condition. The duration of the disease seldom exceeds three months. Castle (2) reports a case which died fourteen days after the onset of symptoms, while Ribadeau (19) reports a case which lasted for four years.

Although a very malignant disease, surgical intervention has met with some success. Schlimpert (22), Keen (14), Williams (29) and Freeman (6) have reported operations for this condition in which the tumor was removed and the cavity curetted or in which the whole affected lobe was successfully removed. Yoemans (32) reports an operation for a recurrence seven years after the removal of the primary growth.

Since Hanot and Gilbert (8) divided primary carcinoma of the liver into three groups, *cancer massif, cancer nodulaire*, and *cancer avec cirrose*, there have been a number of classifications advanced. Goldzieher and v. Bokay (7) divide primary liver cancers into two main groups: (1) those derived from the small bile ducts or "carcinoma cholangiocellulare," and (2) those derived from the liver cells or "carcinoma hepatocellulare." Yamagiwa (31) simplifies this terminology, calling the two types "cholangioma" and "hepatoma" respectively. This is probably the most logical basis for classification. Ewing (5) accepts these terms but classifies under them both benign and malignant epithelial neoplasms arising from the cells of the small bile ducts and from the liver cells.

A number of criteria have been advanced for the differentiation of hepatomata and cholangiomata. Ribbert (20) says that the malignant adenomata which he considers derived from liver cells are usually colored by bile pigment within the cells. Eggel (4) lays great stress upon the morphology of the cancer cell itself, but because of the similarity between the cells of the two classes in certain cases this criterion is limited, for while cancer in many cases conforms to the structure from which it is derived, in other cases this does not hold true.

Wegelin (27) concluded that the liver cell cancer has a stroma composed only of a network of capillaries. The bile-duct-cell cancer has a stroma of relatively abundant connective tissue.

Adelheim (1) agreed with Wegelin (27), and by using preparations stained with silver nitrate showed that the supporting framework in liver cell carcinoma possessed striking similarities to that of normal liver tissue.

The combined statistics of Eggel (4), Yamigiwa (31), and Goldzieher and v. Bokay (7) show that 87 per cent of the cases of hepatoma are associated with cirrhosis, while cirrhosis is found in 50.6 per cent of cases of cholangioma. Every possible hypothesis regarding the relationship of tumor formation to cirrhosis has been advanced. Because the most cirrhotic areas in their material were free from cancer, Kelsch and Keiner (15) concluded that the two processes were entirely independent.

The finding of some other morbid condition in the liver associated with cancer led some investigators to suggest that a disease such as syphilis might be the cause of both the malignancy and the cirrhosis. Wegelin (27) pointed out the possibility of the cirrhosis being secondary to the tumor formation. His view was that, due to its toxic effect, the cancer caused the degeneration of the liver cells with the subsequent formation of scar tissue. The theory that cirrhosis precedes the cancer in the vast majority of cases and is the direct cause of the neoplastic growth was first advanced by Sabourin (21). Schmeiden (24) regarded the hyperplastic liver cell islands which occur in cirrhosis as pre-cancerous stages. The view of Ribbert (20) that cancer arises from liver cells which have become displaced and surrounded by cirrhotic connective tissue, although particularly applicable in this case, is not adhered to by the majority of investigators. The more popular view is that, on account of injury, there is a destruction of liver tissue and that this is followed by fibrotic changes and regeneration. Regeneration leads to the production of hyperplastic nodules of liver cells. These hyperplasias are to be regarded as an expression of repair such as occurs in cirrhosis. The blastomatous degeneration expresses itself in an excess of growth beyond the margin of the processes of overgrowth and regeneration.

As early as 1878 Schuppel (25) described transitions of liver cells to cancer cells at the periphery of the innumerable tumor nodules, and it was generally conceded that each nodule arose in the place where it occurred from the pre-existing liver cells of that area. v. Heukelom (11) showed not only that the columns of liver cells were in many instances directly continuous with columns of tumor cells, but that in many cases the liver capillaries were in direct continuity with the tumor capillaries. From his observations v. Heukelom (11) concluded that primary liver cancer is multicentric in origin, and that there is a progressive metamorphosis of liver cells to tumor cells at the periphery of the growth. These observations have been repeatedly confirmed. v. Heukelom's (11) interpretation has been adopted by Milne (17), Adelheim (1), Travis (26), Goldzieher and v. Bokay (7), and numerous other investigators.

Heussi (12) observed the same pictures as v. Heukelom (11), but by staining with Orange G. the cancer cells and the liver cells were sharply differentiated, and from this he concluded that the apparent connection between tumor and liver cell was an artefact. This view is supported by Ribbert (20) and Herxheimer (10). Ribbert (20) and others have described pictures, similar to the apparent transitions, occurring in metastatic growths where the secondary nature of the cancer was unquestionable.

Regarding the metamorphosis of liver cells to cancer cells, there also is disagreement. Eggel (4) denies the possibility and considers the growth autogenous. Goldzieher and v. Bokay (7) state also that appositional growth does not occur. Wegelin (27), while he admits the possibility of appositional growth, thinks that it seldom occurs.

Adelheim (1), Milne (17), v. Heukelom (11), Goldzieher and v. Bokay (7), and other investigators, who emphasize the above-described transitions, adhere also to the theory that primary liver cancer is multicentric in origin.

The principal advocates for the theory of unicentricity of origin have been Ribbert (20) and Heussi (12). Ribbert (20) says that primary cancer is not necessarily multiple. Small metastases may form very late from the primary nodule. Secondary nodules occur through the invasion of the portal veins by the primary growth. According to this investigator most of the tumor nodules are sections of tumor cords which represent the portal vein dilated and filled with tumor thrombi. In a number of cases he was able to strip the tumor mass from out of the vein in a cast-like form. In other cases there is invasion of the portal vein by the cancer cells followed by embolus formation, and in this way metastases occur to other portions of the liver.

DISCUSSION

Because of the trabecular arrangement of the parenchyma, the occurrence of a stroma composed of capillaries, and the absence of any evidence of proliferative changes of the bile-duct epithelium, this example of primary carcinoma of the liver belongs to

the class of hepatoma, according to the classification of Gold-zieher and v. Bokay, as modified by Yamagiwa.

The picture presented is one of invasion by the cancer cells, and not that of a gradual metamorphosis at the periphery of the cancer nodules from liver cells to cancer cells, as was said to be the case by v. Heukelom. In many instances there is direct continuity between the columns of liver cells and the columns of cancer, the cancer cells growing between parallel pre-existing liver capillaries with the same general arrangement as the liver cells. At the line of apposition, however, there is a sharp de-marcation between cancer cells and liver cells. The liver cell in each case shows marked degenerative and necrotic changes. Nowhere is there hyperplasia of liver cells. This observation can only be interpreted as proving that the so-called transitions of v. Heukelom do not occur in this case and leads to the adoption of the view held by Ribbert, Heussi, and Winternitz, that this growth is autogenous and not appositional. Moreover, in the case of secondary carcinoma of the liver studied, where there was no possibility of transition from liver to cancer cells, pictures identical with those seen in the case of primary carcinoma were observed. It would seem, therefore, that the pre-emption of the liver capillaries by the cancer cells with the degeneration of the liver cells before the advancing cancer, is not restricted to primary liver carcinomas.

The absence of any transition from liver cell to cancer cell would not suggest that the growth had a multicentric origin. In the gross specimen on section, although discrete nodules of cancer tissue may be seen in one plane of section, other planes show that these apparent discrete nodules are cross sections of fingers of malignant tissue which evidently extend from the right lobe in a branching tree-like form. This would lead to the conclusion that the cancer is unicentric in origin, the primary focus being in the right lobe since the bulk of the growth is at that site.

The growth does not seem to have invaded the portal system to the extent of using the veins as a pathway of extension, as was demonstrated in the four cases reported by Winternitz.

In a few of the veins cancer cells are present, and there is a possibility that some of the nodules are metastatic, but from the gross appearance of the tumor it would seem that most of the cancer is formed by direct extension from the primary focus.

As little cirrhosis is present in this case, there could not possibly be any relation between cirrhosis and the causation of this cancer. The small increase of connective tissue occurred after the invasion of the cancer and is only found in the cancerous areas.

SUMMARY

1. On the basis of the arrangement of the cells, the presence of capillary stroma, and the absence of proliferation of the bile-duct epithelium, this carcinoma is classed as a hepatoma.

2. In the case in question, cirrhosis is not present in the liver tissue, and nowhere is there hyperplasia of the liver cells. Although there are numerous instances in which the cancer cells grow between parallel capillaries, and are in direct continuity with the liver cell trabeculae, there are no transitions between liver cells and cancer cells.

3. The growth is unicentric in origin, the primary focus being in the right lobe, from whence it grows by direct extension without using the portal system as a pathway.

4. In the case of secondary carcinoma of the liver studied, appearances very similar to those observed in the case of primary carcinoma are seen.

I am indebted to Dr. Harry T. Marshall for the privilege of using this material and for inspiration in the pursuit of this investigation.

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PLATE 1

Fig. 1. Gross section of left lobe of primary hepatic carcinoma. The lighter striated areas represent the cancerous nodule. The darker background is the relatively normal liver tissue. Two-thirds natural size.

Fig. 2. Gross section of left lobe of liver and attached gall-bladder. Cancerous liver tissue is shown above and to the left. The gall-bladder is shown below and to the right. The gall-bladder shows malignant degeneration. Two-thirds natural size.

Fig. 1

Fig. 2
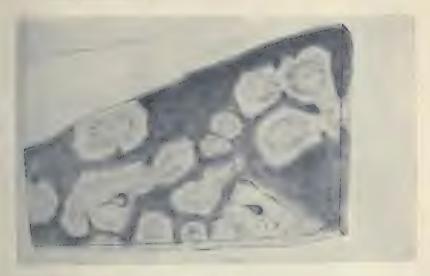
PLATE 2

Fig. 3. Microscopic section of portion of liver showing two adjacent areas
(at left, below) of proliferating cancer cells. The reticulated enveloping tissue
is composed of necrotic liver cells. The connective tissue immediately surround-
ing the two cancerous nodules represents largely collapsed capillaries. This
figure illustrates the advance of the hepatic cancer between parallel capillaries.
Note the multiple mitotic spindle in the central cell of the lower nodule. Magni-
fication 1000 diameters.

Fig. 4. Similar larger area of hepatic cancer cells (above, at left), showing the
advance of the carcinoma between parallel capillaries, and the degeneration of
the liver cells in the path of the encroaching tumor. Magnification 1000 diameters.

Fig. 3
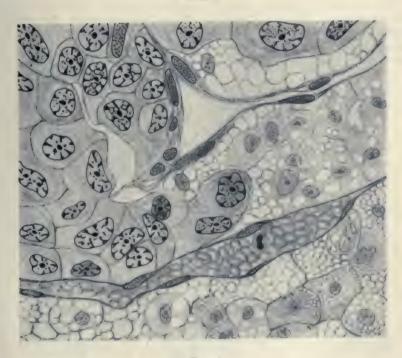
Fig. 4

225

PLATE 3

FIG. 5. Microscopic section from secondary hepatic cancer showing a very mature portion of the neoplasm. Above is a band of dense connective tissue. This band is infiltrated with round cells. To the left and above are groups of hyperplastic liver cells. Below is a nodule of cancer cells. Magnification 200 diameters.

FIG. 6. Area from younger portion of secondary cancer. Above and to the left is liver tissue. Below are trabeculae of invading cancer cells. Two mitotic figures are present in the field. Between the advancing malignant cells and the comparatively normal liver cells is an area of degenerated and necrotic liver cells. Magnification 400 diameters.

FIG. 5

FIG. 6
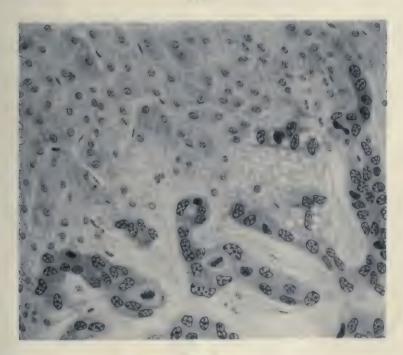
THE EFFECTS OF ROENTGEN RAYS AND RADIO-ACTIVE SUBSTANCES ON LIVING CELLS AND TISSUES[1]

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1. SOURCES OF RADIANT ENERGY WITHIN THE BODY

In estimating the effects of radiant energy in our cells and tissues, we usually leave out of consideration the presence of a considerable source of it within our body. Zwaardemaker, as did others before him, called attention to the fact that potassium, which is an important constituent of certain parts of our tissues, is radioactive; it gives off very penetrating beta rays as well as gamma rays. He believes that potassium, which is required for the equilibrization of body fluids acts, not through its chemical properties, but as a radioactive substance; it can be substituted for by various other radioactive substances, by alpha as well as beta rays. Radioactivity is thus of importance in maintaining the automaticity of the heart action, of the movements of the intestines; it helps to regulate the permeability of the capillary endothelium and thus prevents edema, if acting in the right proportions; it determines also the permeability of the glomerulus for sugar.

This view as to the mode of function of potassium has not been generally accepted. One of the principal objections against it rests on the fact that the functions of potassium may be taken over not only by rubidium, which likewise shows some, although a weak, radioactivity, but, in certain cases, even by caesium, which is not known to give off either beta or gamma rays. Yet,

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[1] In this summary reference is made principally to the more recent literature on the effects of radiation on living cells and tissues.

as R. F. Loeb has shown, caesium can take the place of potassium in a balanced solution which permits echinoderm eggs to develop. Furthermore studies by Hamburger have shown that these functions of radioactive substances are not indispensible, but that a combination of salts, in which a somewhat larger amount of calcium is used than in Ringer's solution, acts equally well, even if radioactive substances are entirely lacking. It is therefore at present still doubtful whether radioactive substances exert a physiological function in the organism.

Lazarus-Barlow found in certain individuals the presence of radiant energy which may be attributed to the taking up of radium emanation. He used the emanation electroscope for its determination and he found this substance especially in the tissues of persons affected by cancer; usually, but not in every case, the cancerous tissue itself was found to be more radioactive than the normal tissues of the same individual. This radioactivity may communicate itself even to gallstones situated in a cancerous gallbladder while stones found in non-cancerous gallbladders are not radioactive. It is difficult at present to interpret these findings; it may be that cancerous tissue of a certain kind is especially liable to retain the emanation. We may also have to reckon with the somewhat more remote possibility that in persons in whom radium emanation is retained in larger quantity, the factors which tend to produce cancer find a more responsive substratum on account of the presence of the radioactive substance. Nothing has to our knowledge been published as to the finding of such a radioactive substance in animal cancers and especially in transplanted tumors.

2. DIFFERENCES IN THE RESISTANCE TO RADIATION OF DIFFERENT TISSUES IN MAMMALS

It is a well known fact that different tissues are unequally affected by radium or Roentgen rays. However, it may be well to consider briefly the various tissues in order to obtain, if possible, an insight into some of the factors which determine these differences in sensitiveness.

The generative organs, ovaries as well as testicles, are among the organs most readily injured through rays. In the ovary it is the larger follicles which succumb first, and only later the small, younger follicles are affected. This is due to the fact that, as we observed, the largest follicles are the most labile structures in the ovary, and as such are most readily injured by a variety of unfavorable conditions as, for instance, lack of a sufficient amount of food and circulatory disturbances preceding the process of ovulation. The medium sized or smaller follicles are less readily destroyed, although mitotic cell proliferation may proceed here more actively, because they are in a healthier, generally more resistant condition. Later, when the granulosa cells of the follicles have become transformed into the granulosa of the mature cells, they have acquired a considerable degree of resistance (Loeb). It would probably be found that such cells are more resistant also to the effect of rays; they resemble the corpus luteum cells, which likewise are relatively hardy. In the testicle it is the actively dividing cells, the spermatogonia and spermatocytes, which are more sensitive, while the resting Sertoli cells and the spermatozoa are much less readily affected, at least as far as the ordinary vegetative functions indicate the condition of the cells.

We learn thus of two factors which determine the sensitiveness of tissues or cells, namely, (1) the intensity of their proliferative activity, and (2) differences in the degree of general sensitiveness, which do not necessarily run parallel to the proliferative activity.

The haematopoietic system is likewise very sensitive to radiation; the lymphocytes especially are a very sensitive indicator of various rays. Even a very small dose of Roentgen rays produces an initial, quite temporary, destruction of lymphocytes. The subsequent increase in the number of lymphocytes which, as Murphy has shown, follows the initial fall, extends over a much longer period of time. Very soft Roentgen rays especially are effective in this respect (Murphy). If larger doses are given the initial fall is followed by a still greater destruction of lymphocytes. The increase in lymphocytes which results from weak

doses of Roentgen rays, is accompanied by an increased multiplication of cells in the germ centers of spleen and lymph nodes, and we may assume that the latter are the source of the increased number of lymphocytes in the circulating blood (Murphy and Nakahara). However, inasmuch as some of these stimulated organs are situated at places far removed from the surface of the body, it appeared improbable that this stimulation should be due to the direct action of the soft Roentgen rays on spleen and lymph nodes. Quite recently Murphy has found an explanation of this effect which is of very great interest. He noticed that, as the result of the exposure to the rays, a substance appears in the blood which, when mixed with lymphocyte tissue in vitro, exerts a stimulating effect upon the latter. The influence of the rays on the lymphocytes is therefore, at least as far as the stimulating effect on these cells is concerned, an indirect one. Lymph nodes and spleen also react by cell multiplication to the parenteral introduction of olive oil and to the application of dry heat to the animal (Murphy and collaborators). The mechanism of this reaction is presumably the same.

Again this sensitiveness of lymphocytes is not limited to Roentgen rays. Lymphocytes are, as our investigations have shown, among the most responsive cells in our body; they finely recognize and react against the most delicate differences in the constitution of various cells and migrate in response to these differences; they are the finest reagent for the discovery of what we have called syngenesio- and homoiotoxins.

Associated with the motor responsiveness of the lymphocytes is the great proliferative activity which proceeds in the germ centers of the lymphatic tissue and which makes them especially sensitive to the effects of raying. The bone marrow is also readily affected by rays, as the introduction of radium emanation into the animal has shown (Bagg). In accordance with this sensitiveness the Roentgen ray treatment of leucaemia is followed by necrosis in spleen and lymph nodes (Warthin). But it seems that in cases of leucaemia the myelocytes and myeloblasts are more readily injured by Roentgen rays than the lymphocytes (Warthin).

The epidermis is very accessible to the injurious effects of the rays, although it is less sensitive than the organs to which we have just referred. Among the appendages of the skin the hair follicles are most sensitive in accordance with their marked proliferative activity, while the resting and differentiated sebaceous and sweat glands are more resistant. It is stated that the mucous membranes are much less sensitive than the skin. This applies perhaps to certain areas; but it seems that the gastric mucosa is easily affected; and Hall and Whipple, as well as Bagg, find certain parts of the intestinal epithelium quite vulnerable to hard Roentgen rays or radium emanation. In invertebrates the intestinal tissue may likewise be relatively sensitive (Congdon). Sometimes the changes found in the epidermis are considered secondary to changes in the blood vessels. This is an erroneous conclusion; both lesions occur independently of each other; yet it is very probable that the thickening and occlusion of blood vessels which may follow radiation can secondarily aggravate the injury of the epithelial elements.

Gland tissues are affected by raying, but with unequal intensity. The parenchyma of thyroid and liver may be replaced by connective tissue. The liver becomes fatty and later necrotic under the influence of radiation of sufficient intensity. The kidney, pancreas, and the salivary and adrenal glands seem to be relatively resistant. In the glands different structures show a different degree of resistance. It appears that the efferent ducts are more resistant than the parenchyma proper, although the former are more primitive, less differentiated, and ought therefore to be more sensitive according to the usual conceptions. This increased resistance of the ducts applies, at least, to the liver and kidney; it is evidently due to the greater general resistance of these structures to various kinds of injurious conditions, and this is a very important factor which helps to determine the special resistance of a certain tissue to radiation.

This greater general resistance, however, is only one factor among several which determine the sensitiveness of tissues to radiation. The central nervous system which as a general rule is very little resistant, for instance to lack of oxygen, is quite

resistant to radiation.   On the whole, cells which produce fibrillar structures outside of the cell proper seem to be relatively resistant.   Radiation of the brain leads primarily to hemorrhages, the vessels of the central nervous system readily giving way, if the walls are injured.   Only very direct radiation of the brain by beta or alpha rays can produce a primary injury of the ganglia cells.   In a similar manner the ganglia cells and nerve fibers seem to be rather resistant to radiation in worms and in crustacea (Congdon).

The very much differentiated striated muscle, as well as fibrous tissue and nerve fibers, are likewise resistant.   The factor of general resistance again explains the great special resistance of cartilage to radiation.   An intensity which elsewhere would produce destruction may stimulate the perichondrium.   However, even in the cartilage intense raying may produce necrosis followed by subsequent proliferation of the perichondrium.

Studies on the resistance of plants to radiation confirm and extend some of these conclusions.   In general, adult plants are more resistant to radiation than animal tissue.   Here also the growing (meristematic) tissues are more sensitive than the relatively quiescent parts.   But again there is no absolute parallelism between proliferative activity and sensitiveness to rays.   There is added to the sensitiveness of proliferative tissues a particular sensitiveness of certain parts which may show less proliferative power than other less sensitive parts. In certain cases parts of plants near the growing tip, which are relatively rich in fat and poor in starch, are especially accessible to the necrotizing effects of the rays.

In the case of buds the sensitiveness increases with their increasing activity.   And when at an early stage the growth of the bud is just beginning but as yet proceeding at a slow rate, a dose of rays which in the resting stage of the plant would be quite ineffective and in the actively growing plant injurious, may have an intermediate effect which shows itself in stimulation.   Thus it is possible to hasten the development of winter buds through raying (F. Weber).

Resting plant tissues like dried seeds and spores, are very resistant to radiation, although they are not altogether inaccessible to the effect of the rays. Increasing their state of hydration, even without an accompanying increase in growth processes, increases their sensitiveness, but to a much lesser degree than does the process of germination. Drying the germinating seeds diminishes again somewhat their sensitiveness to raying, but the effect of the growth processes, which latter tend to diminish the resistance of the tissues, by far overbalances that of the state of hydration. In a parallel way hydration renders proteins more sensitive to the effect of heat. Again we thus have in this case not to deal with a specific effect of the rays.

Of greater significance than either hydration or development may be the structural or chemical differences which are found in the seeds of different species and which may cause a very great difference in the resistance of various seeds (Petry). On the other hand, suppression of oxidation processes and the accompanying reduction in metabolism does not necessarily lead to a reduction in sensitiveness (Petry). Certain physiological activities may not only not lower the resistance to radiation, but on the contrary increase it. Both Wellcock and Packard found that algae or animals, serving as hosts of photosynthesizing plants, show more resistance to radiation in the light than in the dark. Somehow the metabolic activities associated with photosynthesis counteract or prevent the injurious effects of the rays.

The fact that growth rate is not the only factor which determines the sensitiveness towards radiation comes out still more strikingly in the case of the infusorium paramaecium. This organism is most resistant to the effect of rays during the middle period of its cycle, in which cell divisions are most frequent; yet at this period the general vigor of the organism is at its height, and it can therefore resist the deleterious effects of rays, as well as other injurious influences, better at this time than at the first and third periods of the cycle, when the proliferative activity is lessened, but when at the same time the general vigor is lowered (Markovitz). The unfavorable effects of radi-

ation and the constitutional weakness prevailing at this time of the cycle produce thus an additive injury. In general it has been found that the resistance of protozoa to radiation varies greatly in different species.

We have mentioned that the effect of radiation on seeds varies in accordance with the species to which they belong. In a similar manner Nereis eggs are found to be much more resistant to radiation than sea urchin eggs (Packard); different species of bacteria and infusoria differ likewise in thier resistance to raying. In these cases we must assume that the chemical and structural difference between the species determines the difference in their resistance. In the case of skin, however, we may in all probability refer species differences observed to variations in proliferative activity. Thus it has been found that the skin of the guinea pig is more sensitive to radiation than the skin of the rabbit. This we may provisionally attribute to the greater proliferative activity which, as we found previously, is characteristic of the epidermis of the guinea-pig.

In accordance with the stronger effect of raying on less differentiated and more actively dividing tissues, embryonic tissues should be expected to be more sensitive to the effects of rays than adult tissues, and this is actually the case. This fact comes out very clearly when pregnant females are exposed to the influence of rays. In Daphnia the embryos are killed by a dose which leaves the mother intact; only if the dose is increased does the mother become sterile, while a still larger dose may kill her. Similar results were obtained by Bagg, when he introduced radium emanation into pregnant rats or into rats preceding fertilization; the embryos were affected under conditions which left the mothers apparently normal. But if a considerable dose of emanation was injected into the animals, the mothers also showed symptoms of toxemia and lesions in various tissues. In accordance with the size of the dose given, different degrees of injury could be obtained in the offspring. In cases of light injury the young were born in an apparently good condition. Only at autopsy, a considerable time after birth, a retardation in the development of the cortex of the brain was observed. In

slightly more severe cases the young developed eye defects, but otherwise were normal. These effects were observed when filtered emanation was applied over the abdomen of the mother towards the end of pregnancy. In case emanation was injected parenterally the young were more severely affected by the emanation, which passed through the placenta and caused lesions of the blood vessels, hemorrhages, edema, and changes in the liver and intestines.

Radiation of the bird's egg led in some cases to the destruction of the embryo proper, while the embryonic membranes developed. This indicates a greater power of resistance of the latter structures, an observation which agrees with the greater resistance of the embryonal placenta as compared to the embryo proper, which we observed in ova developing parthenogenetically in the ovaries of guinea-pigs. Again we have to deal with a general resistance and not with a particular one to radiation.

The effect of the rays acting on the embryonic cells directly depends (1) on the intensity of the radiation used; (2) on the stage at which the developing embryo is exposed to the rays; and (3) on the sensitiveness of the particular species used. The more intense the radiation and the earlier the radiation is applied, the more marked are the effects. Very weak quantities may under certain conditions accelerate the development slightly, stronger quantities may merely retard the development without causing monstrosities. Still stronger quantities cause faulty development. The radiation affects in the first place the development of the eye, the nervous system, and the myotomes. Stockard assumes that these structures are affected most readily because they enter into their critical phase of growth and differentiation later than other structures, namely, at a time when the effect of radiation can make itself fully felt. On the other hand, it is conceivable that these differentiations require the finest adjustments and that while the derangement caused by radiation is unable to affect seriously coarser adjustments, it must interfere with these more delicate mechanisms, just as in the adult organism the generative organs are more readily interferred with than others. We see then that while the adult differentiated nervous

and muscular systems are quite resistant to the effect of rays, the undifferentiated nervous and muscular systems of the embryo are among the most sensitive tissues.

If the raying is done with still greater intensity and at still earlier stages, the embryo dies at an early stage of development. An interference of graded intensity with the development of the embryo can be produced through raying of the spermatozoa even if it should not be strong enough to interfere with the motility of the latter (Bardeen, Hertwig), or likewise through the raying of the ovum before fertilization. All degrees of interferences in development can thus be produced. The radiated chromatin cannot function normally and interact healthily with the chromatin of the partner cell, and it in some way acts as a poison on the latter, which had not been radiated previously and was therefore normal, before the changed chromatin had a chance to act on it.

In this connection Hertwig observed a paradoxical phenomenon in the radiation of eggs and spermatozoa of amphibia. It was found by Hertwig that a more intense radiation of egg or spermatozoon may, under certain conditions, permit a better development of the embryo than a milder exposure. This result is due to the fact that a more severe radiation still permits the rayed chromatin of the spermatozoon to cause a parthenogenetic development of the ovum, but at the same time injures the spermatozoon to such an extent that it is unable to interfere with the healthy egg nucleus which, in consequence, alone directs the development of the embryo. If in the converse experiment the egg chromatin has been eliminated through a more intense radiation, it is the healthy sperm chromatin which induces a merogonic development of the embryo. In case the radiation has been less intense, the injured chromatin is able to attach itself to the normal chromatin and to impede the activities of the latter. While the parthenogenetic or merogonic larvae are superior to those produced through a combination of normal chromatin with less intensely rayed chromatin, yet even the former are by no means normal. In a somewhat similar way it may be possible to neutralize through radiation to some extent the injurious

effect of the fertilization of an egg with a spermatozoon of a different species. In this case the rays can apparently destroy that part in the chromatin which determines the species character; they may thus affect what we have designated as "hetero-differential." If the radiation injures at the same time the chromatin of sperm as well as of ovum, the effects of the radiation are much more severe than if only one component of the fertilization nucleus has been affected.

The same factors which tend to make embryonic structures more sensitive to radiation act similarly in the case of regenerating tissues. Regenerating cells are more rapidly dividing than normal cells, and in addition they usually show a more simple constitution, the paraplastic structures developing only at later stages. Thus the regeneration of the tail of the tadpole is readily inhibited by radium (Schaper). The process of healing in a skin wound is likewise retarded by radiation. The movements of the epithelial cells are inhibited and inflammatory processes are called forth in the granulation tissue; or if the action is more severe the margin of the epithelium is destroyed and the formation of granulation tissue is likewise suppressed. At a place further distant from the rayed area the epidermis may on the contrary be stimulated (Werner). According to Werner, radiation of the skin before a wound is made, likewise retards subsequent healing, while the scar tissue which forms after healing is more resistant to the effects of radiation.

3. EFFECT OF RADIATION ON NUCLEUS AND CYTOPLASM

Some observations mentioned in the preceding chapter indicate that it is primarily the nucleus of the cell which is affected by radiation. Earlier investigators, like Perthes and Bohn, noticed changes in the nuclei of dividing eggs as the result of radiation, and they concluded that the rays act mainly on the chromatin of the cells. The subsequent experiments of Bardeen, and especially of Oscar, Günther and Paula Hertwig, made this conclusion extremely probable. Further evidence was produced by Mottram, Oppermann, Payne, Packard, and particularly by the recent experiments of Mavor.

The principal evidence in favor of this conclusion is as follows:
(a) If a female Drosophila is exposed to Roentgen rays at a time
when her eggs undergo maturation, the chromosomes may fail
to separate properly and a subsequent fertilization may lead to
the production of some individuals which show an abnormal
distribution of hereditary characters. The results thus ob-
tained can be interpreted on the basis of Morgan's theory of the
location of hereditary factors in different chromosomes. (b)
Separate radiation of the sperm and of the egg preceding fertili-
zation leads, in various species, to fargoing changes in the be-
havior of the chromatin. The rayed chromatin either fails to
move towards the other chromatin, or it unites with it after
some delay; sometimes it unites not with the egg nucleus, but
with the chromatin of a blastomere. Often the union of the
normal with the rayed chromatin is only of a temporary charac-
ter and subsequently the injured chromatin is eliminated. If the
injury of the chromatin has gone still further, fertilization occurs,
but the chromatin forms clumps and plays no further part in devel-
opment. Now, the spermatozoon contains principally chromatin,
while in the egg both nucleus as well as cytoplasm constitute the
cell. Yet the effects of radiation are very similar in egg and sper-
matozoon and we may therefore conclude that the factor which
both have in common, namely, the chromatin, has been primarily
affected by radiation. The effects of the union of radiated
sperm with normal egg, or vice versa, resemble the effects ob-
served in heterofertilization. In both cases we have to deal with
a union of two kinds of chromatin which are incompatible with
each other. However in the one case the incompatibility depends
on the preformed constitution of the chromatin, in the other
the incompatibility has been produced experimentally. (c) If
the fertilized eggs are radiated, very fargoing changes in the
chromatin may become noticeable; pathological changes in the
mitotic division occur, sometimes the chromosomes clump, or
karyolysis occurs. In the dividing egg of Ascaris the chromatin
may be dispersed into small particles. (d) Radiation is followed
by much more serious results, if it is carried out during the
mitotic division of the cell (Mottram). The resting nucleus is

much more resistant to the effects of radiation than the nucleus during the early stages of mitosis (Richards). In the period of the metaphase the cell seems to be especially sensitive (Mottram, Packard). During mitosis fine adjustments take place in the cell and particularly in the nucleus. However, in different species different parts of the nucleus seem to be unequally affected; in some the achromatic, in others the chromatic elements are primarily altered. In developing eggs of Ascaris the visible structure of the chromatin of the future germ cells differs from the structure of chromatin seen in the prospective somatic cells (Boveri) and accordingly Payne observed that radiation affects both kinds of chromatin differently. Radiation of tissues may also lead to abnormalities in the dividing nuclei. Amitosis and the formation of giant cells and plasmodia may become noticeable. However, these effects are not characteristic of radiation and may be found whenever an abnormal proliferative stimulus reaches an otherwise normal cell which is capable of dividing, or when a normal proliferative stimulus reaches a cell which shows abnormalities in its structure and function. (e) In paramaecia radiation produces nuclear changes comparable to those taking place during endomixis (Markovits); it accelerates in these organisms nuclear division or fragmentation.

While these observations show the importance which is to be attributed to nuclear changes in interpreting the effects of radiation, they do not prove that the effects on the protoplasm are negligible. On the contrary, there are indications that the cytoplasm may be affected very readily by raying. Thus in the egg of Nereis radiation may profoundly alter the surface layer of the egg and thus allow multiple spermatozoa to enter the ovum (Packard). When unfertilized Nereis eggs are exposed to radiation from radium and then fertilized, the fertilization membrane which results is of unusual thickness (Packard), and a quantitative relation of a certain kind exists between the intensity of radiation and the thickness of the membrane (Redfield and Bright). Definite cytoplasmic effects have been observed also in other eggs. If the injurious action is still more intensive, processes of solution or condensation are observed in the cyto-

plasm. Thus a liquefaction may occur in the egg of Nereis. In paramaecium, on the contrary, Markovits observed at an early stage following radiation a condensation of the cytoplasm. In tumor cells a swelling of nucleus and cytoplasm with vacuolization of the latter has been noted by various authors (Ewing, Alter). It is probable that the permeability of the surface layer of the cell is altered as the result of radiation; at the same time the osmotic pressure, in all probability, undergoes changes in the cells and thus water is taken up and solution or vacuolization occurs. Whether the condensation is a secondary change or whether it may be primary, is not certain.

We may then conclude that radiation may affect the nucleus at a very early stage and that the most far reaching changes caused by radiation are probably produced through alteration of the nucleus. But it appears very probable that finer changes may also occur at an early period in the cytoplasm as a result of the raying. In many cases the latter changes are perhaps not yet morphologically discernible, as little as are the finest changes in the nucleus at once recognizable structurally. The nucleus, on the other hand, is a very sensitive reagent which readily indicates chemical changes through alterations in hereditary properties in differentiation and growth processes. Finer alterations in the cytoplasm are apparently of much less importance and do not necessarily lead to as far going consequences as slight changes in the constitution of the chromatin. The criteria which we use in the case of the cytoplasm are of a cruder nature; they depend upon coarser morphological characteristics and on changes in motility. The motility of a spermatozoon may still be normal at a time when the finer chemical composition of its chromatin has already been markedly affected.

There is a series of changes produced by radiation in the various functions of the cells. We can grade this series according to the amount of raying necessary to produce the respective changes. The smaller the amount of radiation is, which is necessary for the purpose of interfering with a certain function, the more delicate is the character of this function. Apparently the most delicate mechanism is the one which determines the transfer of

hereditary characters; it may be disturbed without any other alterations being noticeable. The rate of growth and the differentiation of certain sense organs and of certain parts of the central nervous system and of the myotomes is also readily influenced. The multiplication of less differentiated cells may still proceed, until the stage has been reached when the more delicate organs differentiate. Yet in general, cell division is more readily retarded than the motility of cells and their oxidative processes.

. These investigations make very improbable the view that radiation acts mainly by decomposing lecithin; it can be shown that the effects of radiation are independent of the content in lecithin in various kinds of cells.

4. THE EFFECT OF RADIATION ON TUMOR CELLS

In general rapidly growing tumors are more sensitive to radiation than the majority of other tissues; thus excised pieces of tumor tissue, when exposed in vitro to the action of rays, are more readily injured than, for instance, leucocytes when exposed to the same kind of radiation. As Regaud especially has shown, among the tumor cells those cells which are in the process of mitotic division are particularly sensitive to the action of radiation. However, according to Regaud it is probable that the resting cancer cells also are more sensitive to radiation than the resting cells of the corresponding normal tissues, although definite evidence tending to support this conclusion has, as far as we are aware, not yet been published.

The morphological changes which radiation produces in tumor cells have been studied most minutely in human cancers which have been exposed to the action of radium (Ewing, Alter). The destruction of the cancer cells is often preceded by irregularities of mitotic division, formation of giant cells and nuclei (Clunet, Russ and Chambers), by a cessation of mitotic activity, by a swelling of the nucleus and cytoplasm, or by pyknosis and vacuolization of the cell, evidently due to the taking up of water by the cells and to processes of solution. It has been suggested that the vacuolization is the result of a rise in osmotic pressure

within the cells, which presumably would be caused by the breaking down of larger molecules into smaller ones. It is however possible that changes in the hydrogen ion concentration take place within the cell as a result of radiation and that this may increase the water-binding power of the proteids. The cancer cells which disappear are supplanted by growing connective tissue which, accompanied by blood vessels, invades the parenchyma and at first actively subdivides it and in the end replaces it. This connective tissue may in the beginning be rich in fibroblasts, eosinophiles, lymphoid and plasma cells, but soon dense fibrous tissue is produced. Whether this activity of the connective tissue is entirely secondary to changes in the parenchyma and caused by the latter, or whether in addition there is a direct stimulating effect of the radiation on the migratory activity of the connective tissue, is uncertain at present. Usually the disappearance of the tumor cells goes hand in hand with the substitutive action of the connective tissue. There may be also a direct invasion and destruction of cancerous tissue by connective tissue cells and lymphocytes (Ewing). Under certain conditions radiation causes a less acute degeneration which proceeds in a manner similar to that found in certain normal tissues living under unfavorable conditions. Thus in squamous cell carcinoma keratinization and pearl formation may be promoted (Alter). Radium accelerates in this case a differentiation of a degenerative character. Or relatively inactive structures, not unlike those found in analogous normal tissue may be produced. Thus in squamous cell carcinoma, cysts lined by stratified epithelium may be produced; in adenocarcinoma structures resembling resting glands are occasionally found as the result of radiation (Alter). Earlier authors also had noticed, under the influence of radiation, a transformation of tumor structures, characteristic of a very rapid growth, into structures which are found in more benign, less rapidly growing tumors.

The effect of radiation on cancer tissue varies in different kinds of cancer. Basal cell carcinoma yields much more readily to radiation than squamous cell carcinoma; the cells of the basal cell carcinoma are destroyed by radium under conditions to which

the cells of a squamous cell carcinoma are resistant or which induce in the latter differentiation of a degenerative character. This difference in the behavior of these two types of carcinoma cannot be due to differences in the kinds of cells from which they develop, both taking their origin from the basal cells of the epidermis; but in the case of the one there is a tendency of the cells to differentiate, to produce paraplastic structures, while in the case of the other the cells remain relatively simple and undifferentiated.

Adenocarcinoma is more sensitive to radium rays than squamous cell carcinoma; but its situation deep in the tissues protects it to some extent and may simulate a greater resistance (Ewing).

Seitz and his collaborators have published very definite data as to the comparative resistance of various tumors and normal tissues to very penetrating Roentgen rays. They take as the standard unit a dose sufficient to cause a certain degree of erythema of the skin. They find: Destructive dose for carcinoma = 100 to 110 per cent of the erythema dose of these authors (carcinoma of the ovary may be more sensitive); sarcoma (and particularly myosarcoma), 60 to 70 per cent of erythema dose.

	per cent of erythema dose
Stimulating dose of cancer	35–40
Myomata	180
Lesions in the intestines and blood	135

According to these data sarcoma is more susceptible to radiation than carcinoma. However, fibrosarcoma is more resistant in accordance with the increase in paraplastic substance present in this tumor. Otherwise the different kinds of sarcoma show approximately the same sensitiveness. In this connection it may be of interest to recall our earlier experiments in 1901 in which we exposed transplanted rat sarcoma to repeated doses of Roentgen rays without observing any noticeable retardation of growth; neither did transplantation of radiated tumors into other rats yield tumors that differed in their growth from control pieces. In these early experiments the intensity of radiation had evidently been too weak.

Similarly Seitz assumes that the different varieties of carcinoma also show approximately the same susceptibility to radiation. The stimulating dose is, according to Seitz, somewhat more than one-third of the destructive dose. Myomata are very resistant in accordance with the relatively considerable resistance shown by normal unstriated muscle tissue. The characteristic power of resistance of normal tissue is therefore preserved in benign tumors of the corresponding type. All of these conclusions have however not been generally accepted and F. C. Wood and Prime, in agreement with a number of European investigators, deny that there is a fixed destructive dose for carcinoma and sarcoma. Even for the same type of tumor the dose may vary in individual cases; while some tumors require two, others require as much as eight erythema doses and there is, according to Wood, no essential difference in this respect between carcinoma and sarcoma.

The preponderating evidence which we have at the present time is in favor of the conclusion that the sensitiveness of different kinds of sarcoma and carcinoma differs widely. Nevertheless, the data on hand permit us to arrange in a tentative manner and with certain reservations the various tumors as to their radiosensitiveness. Tumors composed of actively dividing cells, which are devoid of paraplastic structures, are most sensitive to radiation; this applies particularly to lymphosarcoma in accordance with the great radiosensitiveness of its normal prototype, the lymphocyte. Tumors consisting of cells which are essentially protoplasmic material with embedded nuclei are likewise sensitive to radiation, even if the cells are not very actively multiplying (for instance, basal cell carcinoma, perhaps myeloid sarcoma of bone). Tumors tending to produce paraplastic structures are more resistant, even if they show a certain degree of proliferative activity (for instance, keratinizing squamous cell carcinoma, fibrosarcoma). Resting tumors with much paraplastic substances may be very resistant (myomata, fibromata); the retrogression of myomata following the use of penetrating Roentgen rays usually is an indirect effect of radiation and results from retrogressive changes in the ovaries.

However, it is possible that in addition another factor is involved in the variability which is found in the power of resistance of various tumors to radiation. Apart from their structural peculiarities and their proliferative activity, which makes them more vulnerable to the action of the rays, there may be at work a factor of individual resistance.

In the case of paramaecia we emphasized the fact that these organisms show a greater resistance during that phase of their cycle when the mitotic activity is at a maximum; and we stated that in this case the maximum proliferative activity is an index of their great vitality; the latter, tending to increase their resistance to radiation, thus more than overbalances the effect of greater mitotic activity, which as such would tend to make them less resistant. Now it is possible that in the case of tumors this factor of increased vitality, which goes hand in hand with an increased proliferative activity, may likewise cause a greater resistance to radiation; again in this case we would have to deal with two opposing factors and this condition, in combination with the factors mentioned above may, in part at least, explain the great variability in the resistance of different tumors to radiation.

The individually varying general vigor of cancer cells, as a factor in their power of resistance to radiation, is furthermore indicated by the greater tendency of the more centrally situated tissue to succumb first. We know that generally those tissues are prone to die first, under unfavorable conditions, which are farthest removed from the sources of oxygen supply and nourishment.

5. QUANTITATIVELY GRADED EFFECTS OF RADIATION UPON TISSUES AND TUMORS

We have discussed the general sensitiveness of various tissues and tumors to radiation and the manner in which the rays injure normal tissues in different states of growth as well as tumors. We shall now consider the relation between quantity of rays used and the effects of radiation on living tissues and tumors, and

we shall in this connection refer especially to the effects of rays on cells living in vitro.

In earlier investigations, the writer, in association with M. S. Fleisher, E. D. Corson-White, and O. Ishii, has shown that through exposing pieces of carcinoma or sarcoma in vitro to the graded effects of heat, a graded decrease in growth energy intermediate between full vigor and cell death can be produced. All degrees of this intermediate state can be obtained at will. If the action of heat has been very marked, the previously heated cells soon die after transplantation into an animal. If it has been somewhat less severe, the cells begin to grow weakly after a long period of latency; but after a temporary slow growth, they become stationary or retrogress. A slightly lesser degree of injury leads after transplantation to a somewhat more vigorous growth, which however is still much below the average. The latent period is prolonged, but less so than in the preceding case. The tumor may continue to grow rather weakly or retrogress after some time. In other cases a gradual recovery takes place, which becomes accelerated through further transplantations into new generations of animals. In other cases the transplantation may be continued through a number of generations, during which the growth energy remains depressed. After a number of generations the tumor either begins to retrogress and disappear, or it recovers and regains its full growth energy. A tumor transplanted during the later stages of retrogression usually dies after retransplantation. It is not possible through serial heating of recovered tumors to obtain a cumulative effect of heating, nor is it possible thus to produce an active immunity of the tumor cells. It is doubtful whether the tumor could be kept permanently at this intermediate stage of decreased growth energy. Ultimately it seems either to die out or to recover.

After transplantation of pieces of tumor in which the growth energy has been experimentally decreased, the mitotic proliferation of the tumor cells is diminished and the connective tissue has a tendency to form a dense capsule around it and to invade it. However, the lymphocytes do not play a prominent part

around such tumor pieces. While the fibrous tissue may contribute to the limitation of growth in these tumors, the direct depression of growth energy in the exposed cells is the primary factor; added to this is perhaps a process of immunization in the host animal. We have shown that in these experiments heat produces a diminution in the growth energy of the tumor cells and that we have not merely to deal with a diminution in the number of tumor cells. An "all or nothing" law does not hold good in these cases. The same considerations apply to the effect of Roentgen rays and radium rays on cells and tissues. These agents also may bring about a graded reduction in the growth and metabolic activity of the individual cells and tissues and it does not act merely by reducing the number of cells which otherwise would grow in full vigor.

The results obtained after exposing pieces of animal tumors in vivo (Contamin) or in vitro to the action of radiation are also in other respects very closely comparable to those previously obtained by us through applying heat. The experiments of Russ and Chambers, Wedd and Russ, and of Prime and Wood have shown that all degrees of intermediate growth energy may be obtained through exposing the tumors in vitro to the action of radium. The effect of weakening may continue through a number of transplantations and at last the tumor may die or, if injury has been more severe, it dies directly after a brief period of latency, which latter may comprise approximately the first week after transplantation. In other cases a recovery takes place, especially after a second transplantation (Wood). As in the case of heating, tumors growing with decreased growth energy as a result of radiation, may likewise be enveloped in a dense fibrous tissue capsule. It has been stated that in addition to connective tissue, lymphocytes may invade such pieces of tumor following transplantation. There is, however, apparently one important difference between the tumor tissue weakened through heat and through radiation. While the former, at least in our experiments, as well as in the subsequent ones of Lepper, has no immunizing properties, the latter, according to Wedd, Morson and Russ does have a certain immunizing power.

The behavior of tumor cells under the influence of raying is in certain respects paralleled by the behavior of other kinds of cells. As we stated above, embryos may be retarded in their growth through the action of rays. After a first stage of slowing, they may die. Radiation may bring about merely a retardation of development or, if the effects are still furthergoing, the retardation may be accompanied by abnormality of development (Packard). Chromogenic bacteria may, as the result of radiation, temporarily lose their power to form pigment, but it may return after several reinoculations of the bacteria. Prime observed that under the influence of weak radiation in in vitro cultures of the chicken heart muscle, a retardation may occur in the movement of cells into the culture medium, while stronger doses inhibit it altogether. Furthermore the migration of cells may be inhibited by doses of radiation which still permit the pulsation of the heart muscle cells to continue in vitro.

6. STIMULATING EFFECTS OF RADIATION

It has been found by numerous investigators that intensities of radiation weaker than injurious quantities may stimulate cells and tissues to increased metabolism, as exemplified in the greater production of carbon dioxide; they may call forth increased cell movements and increased proliferation, either of a normal or abnormal character, while a quantity intermediate between the stimulating and retarding one may be nearly ineffective. We shall mention some of the observed stimulating effects.

Cell activity is intimately connected with fermentation. Now it is of interest that several investigators state that proteolytic, as well as other ferments, may under certain conditions be stimulated through radiation. The increase of autolysis observed by Neuberg in radiated tumor tissue was referred by him to an increase in the activity of autolytic ferments. Richards in particular concludes that in the case of pepsin and diastase the same relations hold good as in the case of cell activities; in both cases the intensity of radiation determines whether the effect shall be stimulating or inhibiting.

Stimulation has also been observed in plant tissues. Radiated seeds may show an increased production of carbon dioxide (Redfield and Bright); germinating seeds may grow faster under the influence of radiation (Gager, Koernike). The rest period of winter buds may be shortened through radiation (Weber); radiated buds may show an increased growth (Molisch). In radiated root tips the number of mitoses may be increased. In ova Bohn effected the first stages of parthenogenetic development through radiation. Weak doses may accelerate the first segmentations of eggs (Lazarus-Barlow, Richards, Packard).

In paramaecia the first division following exposure to mesothorium is usually delayed, the following divisions, however, are accelerated (Markovits). Radiation of developing eggs (in amphibia, birds, Drosophila) may first accelerate development; this is usually followed by retardation and malformations (Gilman and Baetjer, Congdon). In chick embryos the directly exposed ectoderm may show atrophy, while in the entoderm increased development takes place. Weak intensities of radiation may stimulate the regeneration of tubularia (Congdon). In certain cases pieces of animal tumors radiated in vitro show, after transplantation, increased growth energy which is, however, limited in duration (Prime, Kimura).

Roentgen rays acting on pieces of tumor growing in tissue culture may increase the production of carbon dioxide and the energy of migration in the case of sarcoma, and the production of carbon dioxide in the case of carcinoma. Stronger doses diminish the production of carbon dioxide and cause suppression of division (Kimura). Price-Jones and Mottram likewise observed in vitro a prevention of mitotic division under conditions which permitted an unimpaired migration of the cells. In adult animals or man increased hair growth has been attributed to radiation. Around scars which resulted from radiation, there may be increased pigmentation and hypertrichosis. Weak doses of radiation, repeated at suitable intervals, stimulate in certain cases the proliferation of the epidermis (Werner, Rowntree). Radiation may temporarily increase the excitability of the cortex of the brain; this effect is followed by a decrease in excitability.

Radiation is able to increase the excitability of peripheral nerves and prolong the excitability of nerves outside the body (Lazarus-Barlow). We have already referred to the stimulation on lymphocytes and lymphocytic tissues (Murphy and Nakahara) which follows a transitory depression (Mottram, Russ and Chambers). P. Lazarus found that weak intensities of radiation may stimulate the growth of newly born mice and may accelerate the opening of the eyes. Sugiura and Failla also observed that 2 to 4 millicurie hours of radium action accelerate the growth of young mice, while 11.5 millicurie hours are ineffective, and larger doses may be injurious.

It has been found by many clinical observers that intensities of radiation insufficient to inhibit tumor growth may on the contrary stimulate it. This is not a specific effect. We observed previously that various kinds of mechanical stimulation may induce stationary tumors to resume their growth. Radiation of tumors may lead at first to increased mitoses, which somewhat later may be followed by abnormal growth processes, such as amitosis and formation of plasmodia (Clunet, Marie and Raulot-Lapointe). Some authors attribute the stimulating effect on tumor growth of relatively low intensities of radiation, to the hyperaemia which is produced through the radiation rather than to a direct stimulating effect on the tumor cells. Such a conclusion does not seem to be justified, inasmuch as to my knowledge the growth promoting effect of hyperemia on tumors has not yet been demonstrated; it is certainly unnecessary to assume that the stimulating action of radiation on tumor cells is a secondary effect, if we know definitely that various kinds of cells can be stimulated directly by the rays under conditions which preclude the coöperation of hyperaemia.

These observations, to which others might be added, prove that in all kinds of cells small doses of radiation may increase various activities. This effect is, however, invariably temporary with one exception, to which we shall refer later. The stimulation may subsequently be followed by retardation of growth, abnormal development, cell death or necrosis of tissue. Thus, following stimulation of winter buds, certain parts of the buds

may become necrotic. Tradescantia may first be stimulated and then die. Stimulation of the radiated epidermis may be followed by necrosis. In developing embryos stimulation is followed by retardation, to which may be added the occurrence of abnormalities in structure (Gilman and Baetjer, Richards). Hastings, Beckton and Wedd observed that, while radiation of the silkworm causes the eggs to hatch earlier in the first generation, in the second generation a retardation and loss of fertility are noticeable.

We may interpret the association of stimulation and subsequent injurious effects by assuming that the accelerated course of metabolic and structural changes leads to derangements in the life of the cells. Certain regulative processes which are active at the normal rate of metabolism are lacking, when the rate becomes more rapid. The effect of this deficiency is cumulative and becomes noticeable after some time. We know that various kinds of stimulation are followed by a decreased reactivity and a refractory period. Or it is possible that in some of these cases the stimulating effect takes place during the latent period which often follows stimulation, and that during this period a gradual accumulation of radiation effects occurs. When the accumulation of effects has reached sufficient strength, the latent period is over and injurious effects prevail.

But the opposite course of events has also been observed. In paramaecium the stimulation may be preceded by a retardation of cell division. This is attributed by Markovits to a transitory injurious effect of radiation on the cytoplasm. But Bovie has observed a similar sequence of events in paramaecium, namely, an inhibition of division followed by acceleration under the influence of rays given off by the ultraviolet light of the quartz lamp. This author suggests that as a result of radiation a toxic photo-product may be produced, which is gradually removed from the cells and which acts as a stimulant to cell division when the amount becomes very small. The stimulation of winter buds likewise occurs after a latent period. Lazarus-Barlow noted that gamma rays may at first have an inhibiting effect on squamous cell epithelium; when the after effect of the

rays becomes weaker, stimulation occurs.   It is then possible that
this peculiarity of the radiation curve is due to unfavorable by-
effects of radiation which are temporary, or to a gradual weaken-
ing of the effect of raying which at last leads to such a reduction
in the strength of the rays that they become suitable for stimu-
lation.   In the case of the buds the first injurious effect of the
rays may not be noticeable and only after the passing of a latent
period may the stimulation become apparent.

In some cases it is doubtful how far the stimulating effect of
the rays is a direct result of radiation and how far it is an in-
direct result, subsequent to the injury of other parts.   This
applies, for instance, to the growth of the perichondrium in
radiated cartilage.   We have observed very often that the
perichondrium begins to proliferate if the cartilage adjoining it
has been injured.   The same holds good in the proliferation of
epidermis in the neighborhood of necrotic areas, or in the thick-
ening of vessel walls.   These effects may not be the result of
direct stimulation through radium, but a non-specific regen-
erative reaction, which is found generally in the neighborhood of
injured tissue.

Lazarus-Barlow believed at first that radium may have two
different effects, a stimulating and an inhibiting one.   However,
such a combination of stimulating and inhibiting action is not
peculiar to radiation; it applies to all kinds of alterations which
affect living systems.   The latter are so constituted that they
respond to disequilibrations with changes which tend to reëstab-
lish the old equilibrium, provided the disequilibration was not
so thoroughgoing that it led to irreparable injury.   According
to the sensitiveness and the character of the system, the amount
and character of disequilibration which can be repaired varies;
the character of the system also decides what kind of equilibrium
is reëstablished and by what means it is accomplished.   These
means usually imply temporary excess activity on the part of
the system, and this excess activity appears as stimulation.

As the result of often repeated stimulation of tissues which are
able to respond with growth processes, at last a transformation
may be accomplished in which the effects of stimulation are

not temporary, as in the instances we mentioned above, but in which the effects have become permanent; or, expressed differently, in which cancer has been produced.

As the result of the long continued use of Roentgen rays, cancer has been produced in man and in rats (Clunet, Marie and Raulot-Lapointe). In man the epithelial changes are preceded by changes in the underlying connective tissue and in the blood vessels (Wolbach). Carcinoma as well as sarcoma has thus been produced through radiation. We have every reason for assuming that this cancerous transformation is not specific for radiation, but that it is merely an example of the effect of long continued stimulation in general, which may vary in its character in different cases. A long period of apparent latency may precede the complete cancerous transformation. During this period the tissue, step by step, acquires that increase in intensity and permanence of growth and motor activity which characterizes cancer. It is as yet doubtful, how much, in cases in which ulceration is a prominent feature of radiation, non-specific regenerative stimuli may be added to the more specific ones of radiation proper. It is also conceivable that the often repeated action of the stimulating agent may call forth processes of immunization in the affected cells which make them more resistant to the more injurious effects of radiation, and that such relatively resistant cells are a more favorable substratum for this cancerous transformation.

As we have already stated, all other stimulating effects, except those leading to the formation of cancer, are of a temporary character, ending either in a return to the normal condition or in pathological changes. The same temporary character we noted in cases in which such a retardation of growth in tissues and cells was accomplished that an intensity intermediate between normal activity and death resulted. This intermediate condition represents an unstable equilibrium of the cells and tissues and it tends to be replaced by a more stable one, either normal life or death.

7. LATENT PERIOD

The effect which follows radiation becomes noticeable in many cases only after a period during which the radiated tissue is apparently normal. This is called the latent period. It is observed in the case of the radiation of various tissues and of tumors; it is especially well known in the case of skin burns following radiation; but it may occur equally after radiation of eggs, spermatozoa, and embryos, where the effects are delayed the more, the less severe the radiation. It may also be found in the case of tissue cultures of heart muscle (Prime), where the weakening effect of the raying may appear only after several retransplantations in vitro. It has furthermore been observed in the radiation of plants; and a latent period may precede the stimulating action as well as the inhibiting effect of the rays. The length of the latent period seems to vary approximately inversely to the intensity of radiation used. It also depends upon the character of the system upon which the rays act. A smaller quantity of radiation applied to a sensitive sarcoma may be equivalent to a larger quantity applied to a more resistant squamous cell carcinoma (Clunet and Raulot-Lapointe). In case the radiation used is sufficiently strong, the latent period may be absent and the effect becomes noticeable very soon after radiation. These variations in the intensity of radiation used by different investigators and the variations in the sensitiveness of the system upon which the rays have acted, probably explain the variability in the results recorded by different authors, some finding after radiation of eggs a latent period, others noticing the lack of a latent period.

Toxemia may follow very promptly after radiation. Lymphocytes are affected almost instantaneously. The movement and metabolism of cells growing in vitro may likewise show the effect of radiation within a very short time. The changes in the egg, which lead to polyspermia, may take place very soon after the application of rays.

We may therefore conclude that in addition to the two variables named above—intensity of radiation and sensitiveness of the system upon which the rays act—there is a third factor to be

considered.   Certain effects of radiation may become visible at
once or very soon after the raying, while other effects may
appear only after a more or less protracted period.   In the
case of the latter the primary effect also occurs probably
instantaneously; but it is not visible.   This primary effect is
followed by a chain reaction and it is only a later link of this
chain reaction which becomes apparent.   Thus radiation of the
Nereis egg, previous to maturation, is at first apparently without
effect; but as soon as maturation has occurred a thickening of the
egg membrane becomes noticeable (Packard, Redfield and Bright).
We must assume in this case that a chain of reactions was set in
motion by the radiation and that a link which became visible
depended upon the completion of the maturation process.   In
the case of the egg of Ascaris, radiation produces changes in the
chromatin as early as in the first division; yet the segmentation
proceeds normally.   It is only in later divisions that serious
difficulties arise (Hertwig, Payne).   Although the effects of
radiation become visible in this case at an early stage, yet as far
as the lethal effects are concerned, there exists a latent period.
In this case the latent period is perhaps due to a sensitiveness to
the effects of radiation which increases with the progressive
development of the embryo.

In other cases a relatively small primary lesion may lead
gradually to secondary interferences and reactions during the
functioning of the tissue or organism, which aggravate the
effects of the primary lesion and which may at last seriously
interfere with the functioning of the organism and result in
death.   Such chain processes may take place within the affected
cell, or a change in one structure may affect adjoining structures
and here cause secondary changes.   Primary changes in the
endothelium of the blood vessels may be followed by inflammatory
reactions in the wall of the blood vessels, by edema, and lack of
nourishment of the connective tissue and by fibrosis.   This in
turn may aggravate the state of the overlying epidermis, which
was already primarily affected by the radiation.

In the case of radiation of tumors the chain reactions may
extend further and include reactions of the host tissue.   Around

the cancer tissue, weakened through radiation, the host tissue may form a dense fibrous capsule or perhaps a lymphocytic reaction may occur. After transplanting heated tumor tissue, the weakened tumors may grow for some time and then retrogress. In this case we may also speak of a latent period, although on the whole the effects of heat on tumor cells are noticeable instantaneously.

8. THE RELATION BETWEEN INTENSITY AND CHARACTER OF RAYS AND THEIR EFFECTS ON CELLS AND TISSUES

We have repeatedly referred to a relation between the intensity of radiation and the effects of the rays on living organisms. We saw that intensity of radiation and duration of latent period vary inversely, and that different functions of cells are affected with unequal readiness by radiation. The constitution of the germ plasm is readily influenced by small doses. Next in order of sensitiveness we find growth processes. The ameboid movements of cells are somewhat more resistant and the movements of cilia and flagellae (spermatozoa) seem to be quite resistant. If we grade still further one of these factors, namely, growth process, we can establish a consecutive series in the sensitiveness of different functions to radiation. Beginning with the most severe effects, the order is as follows: (1) Cell death without growth; (2) Cessation of growth; (3) Diminution in growth energy, followed by cessation of growth and death; (4) Diminution in growth energy followed by recovery; (5) A zone where radiation appears to be without consequences; (6) Stimulation. A gradation in the effects in accordance with the strength of radiation comes out very clearly in the experiments of Sugiura and Failla. An exposure of young mice to 2.4 millicurie hours accelerates growth; 11.5 millicurie hours are without effect; 21.9 millicurie hours retard growth; 31.6 millicurie hours not only retard growth, but they cause death on the twelfth day; an exposure to 36.5 millicurie hours is followed by death on the ninth day. A similar gradation is found in other objects, such as seeds, plants and tissue cultures and embryos. Kimura, for instance, observed that soft Roentgen rays, according to the dose

administered, affect quantitatively the number of mitoses of tumor tissue growing in vitro; and the growth energy of the developing tumor, after transplantation of the piece into a living animal, corresponds to the mitotic activity exhibited in vitro.

The readiness with which such a gradation occurs in the same tissue varies, however, under certain conditions. Thus it has been stated that in young mice the growth functions of the ovary are either altogether destroyed or left unaltered, while in somewhat older mice an intermediate result of radiation can be obtained, in which a recovery from the effects of radiation gradually takes place (Failla and Sugiura).

In determining the effect of radiation on living organisms, the product of the number of rays of a certain kind and the time during which the rays acts is generally used as the basis of the calculation. It is assumed that a deficiency in the time factor can be compensated for by the number of rays used in a volume unit of tissue and vice versa. This assumption seems to hold good in certain cases. Thus the inhibiting effects of radiation on ascaris eggs are apparently determined by the product of the amount of rays and the time (Lazarus-Barlow and Beckton) and the same rule was found to apply to tumor cells (Wood and Prime) but in other cases this rule does not apply. Thus Lazarus-Barlow found that, if the products of time and millicuries remain equal, an increase in the time factor increases the effect of radiation on squamous cell carcinoma and diminishes the effect in case of columnar cell epithelium. In the case of the testicle, an increase in the time factor likewise increases the severity of the result of radiation (Regaud). In a similar way the thickening of the membrane of the Nereis egg following exposure to beta rays is greater, if the time of exposure has been lengthened, than if the quantity of active radium has been increased (Redfield and Bright).

From a physical point of view alpha, beta, gamma, and Roentgen rays are totally unlike each other. The so-called alpha and beta rays are particles moving with a certain velocity, and this is considerably greater in the case of the beta particles, which are

negatively charged electrons, than in the case of the larger positively charged alpha particles. Gamma and Roentgen rays are supposed to be short ether waves, and the shorter the waves are the more penetrating (hard) they are. Does the effect which these rays exert on living things correspond to the fundamental differences in their physical character? Certain difficulties have to be considered in attempting an answer to this question. In the first place gamma and Roentgen rays, as well as beta rays, call forth in the body the production of secondary beta rays, which have a relatively low velocity and are therefore readily absorbed. Secondly, various kinds of rays are given off simultaneously by the majority of radioactive substances and only rarely the attempt has been made to separate the action of different rays. Nevertheless, the facts so far known justify the conclusion that the differences in the effects of various rays on living organisms are of a quantitative rather than of a qualitative character.

Alpha rays are most powerful physically and have been employed by Rutherford in the experimental disintegration of the nuclei of certain atoms. They are likewise the most effective rays biologically, whenever they actually reach cells or tissues. However, being the largest particles, they are readily absorbed by living as well as by non-living matter, and therefore they reach only the most superficial parts of the exposed tissues, provided they have been able to penetrate the interposed layers of inorganic matter. Their range of action is accordingly very limited. However, their effects are apparent if polonium is used as the source of radiation or if radium emanation is injected directly into an organism, as has been done by several investigators, and more recently by Bagg; or if the unfiltered emanation acts directly on the tissues. The effects of the alpha rays are pronounced, whether they act in the body or on the tissues living in vitro; but in kind, the effects are not very different from those obtained by the use of beta and gamma rays. Even the observations of Beckton and Russ, according to whom alpha rays may cause destructive structural changes in Altmann granules and nuclei in rat tissue exposed in vitro, or in frog tadpoles, while

beta and gamma rays merely injure the vitality of those tissues and organisms without causing structural changes—even these observations do not contradict this conclusion; they merely indicate the greater intensity of the effects of the alpha particles. It has been shown by a number of investigators that beta rays may likewise affect structurally the nucleus at periods, when the nucleus is in a functionally active condition. Even stimulating effects can be obtained by the use of alpha rays acting on sea urchin eggs, in the same way that stimulation of sea urchin eggs can be obtained through beta and gamma rays; but for this purpose the time of exposure to alpha rays must be very much shorter than that to beta rays, and the time of exposure to the latter must be much shorter than that to gamma rays, if the same results are to be obtained (Packard). In a corresponding manner injurious effects on the eggs are obtained much more readily by alpha rays than by beta and gamma rays.

Beta rays injure the tissues much more effectively than gamma rays; this agrees again with the fact that beta rays have a lower velocity and are more readily absorbed than gamma rays. This difference between these two kinds of rays comes out quite clearly, for instance, in the experiments of Bagg on the action of rays on the brain: gamma as well as beta rays cause injury to the endothelium of the blood vessels, and hemorrhages; but in addition beta rays, if they act during a sufficiently long period of time, cause a necrosis of a small area of the nerve tissue directly reached by the rays. Beta rays, which have been deflected and thus separated from other rays by the action of a magnet, inhibit the segmentation of the sea urchin eggs (Packard) in a manner which differs only quantitatively from the more efficient alpha and the less efficient gamma rays. Microscopically visible structural changes in tissue seem to be produced by beta rays only within the living organism, and especially if the tissues have a chance to function during a sufficiently long period following the application of the rays.

Among the beta rays differences exist in regard to their velocity and power to penetrate the tissues. The greater the velocity of beta rays, the less they are absorbed and the weaker is their

biological effect.   Therefore the so-called soft beta rays are more effective than the harder beta rays in their action on seeds (Congdon), on luminescent bacteria (observations in Zwaardemaker's laboratory), on Nereis eggs (Redfield and Bright). The latter investigators have shown that the biological effect of various kinds of beta rays is approximately parallel to their absorption coefficient and their ionizing power.

Secondary beta rays which originate when the beta or gamma rays collide with atoms, especially those with greater atomic weight, are very little penetrating. According to Wedd and Russ, as well as to Wood and Prime, the extraneous secondary beta rays which originate when the primary rays are held back by a metal shield, do not exert a noticeable effect on cells and tissues. Only Congdon noticed a definite biological effect of these rays which he rates quite strong, in accordance with the low velocity of these rays.

As to the gamma and Roentgen rays, their biological effect is much less pronounced than the effect of the beta rays.   Roentgen rays possessing a lower velocity—the so-called soft rays—are more active than the more penetrating gamma rays. According to Chambers and Russ soft Roentgen rays show very much less effect on bacteria than beta rays, while the gamma rays of emanation are quite inactive: similarly Zwaardemaker finds only soft beta rays are efficient against luminescent bacteria; gamma rays are inefficient. Of a similar character are the findings in normal tissues and tumors. According to Wedd and Russ beta rays acting on excised pieces of mouse tumor are much more effective than gamma rays. Corresponding results were obtained by Wood and Prime. They found, for instance, that while 83 mgm. radium which gave off beta and gamma rays killed pieces of tumor in vitro after an exposure of one hour, 100 mgm. of radium which gave off only gamma rays required, under otherwise similar conditions, an exposure of seven hours. Of interest also is the great variability in the biological effects of the gamma rays which these investigators observed and which is in contrast with the uniform effects of the beta rays.   In the case of the Nereis egg, Redfield and Bright likewise found a marked action

on the part of the beta rays, while the gamma rays had only extremely weak effects.

Relatively weak as the biological action of the Roentgen and especially of the gamma rays is, these rays are practically the only ones which can be applied in the treatment of tumors, because they are the only ones which pass through normal tissues without causing serious injury, provided the necessary precautions are taken.

While extraneous secondary beta rays are under ordinary conditions not of importance, it has been suggested by a number of authors (among others, Joly, Barclay, Friedrich) that within the cells or tissues the secondary beta rays are the agent to which the x-ray and the gamma rays owe their effectiveness. This would agree with the fact that the efficiency of the various rays corresponds approximately with their power of ionizing air (S. Russ, Redfield and Bright). At present, however, we cannot regard this relation as proved. It is certain that a definite ratio exists between the biological effects of radiation and the readiness with which the rays are retained (absorbed) by cells and tissues; there is furthermore a relationship between their absorption and their ionizing power. However, it is possible that other molecular or atomic changes are produced in addition by these radiations, which also are proportionate to the absorption of the various rays.

If the various radiations acted primarily through the expulsion of electrons (secondary beta rays) their effect would be similar to the photoelectric effects of direct light, which are likewise believed to be due to a splitting off of electrons and a subsequent ionization of the remaining part of the atom. The action of the radiations on living organisms resembles the photo-electric effect also in another respect. In both cases the effect of the temperature at which the action takes place on the velocity of reaction is very slight, as Lepper, Wood and Prime found in the case of tumors, and Redfield and Bright in the case of the egg of Nereis. In the latter the temperature coefficient was only slightly above one.

We have seen that it is possible, through a quantitatively graded use of radiation, to separate different functions in the cell life; the more sensitive functions (those determining species differential, tissue differentiation, growth) are affected very readily; they may be interfered with under conditions in which the less sensitive functions (amoeboid movement, activity of flagellae, production of carbon dioxide) remain as yet uninjured or are, on the contrary, stimulated. Thus the germination of seeds may be prevented while carbon dioxide production is increased (Redfield and Bright). The fact that radiation may prevent cell division at a time when other functions of the cell are still intact or even stimulated is of special interest. Thus Price-Jones and Mottram and also Prime found that radium may prevent mitotic proliferation of tissues in culture media and at the same time may leave unchanged their migratory activity; or the latter may even be increased under those conditions (Kimura). Halberstädter observed that radioactive substances can prevent the multiplication of trypanosomes without killing them, or without preventing their motor activity. In the experiments of Wassermann, the reducing or oxydizing properties of cells kept in vitro were still preserved at a time when the proliferative activity was suspended. In the case of tumors it is the excess proliferative energy, that which distinguishes tumor tissue from ordinary tissues, which is first affected by radiation; on the other hand the temporary tissue proliferation which may be observed after homoiotransplantation may persist after an exposure to a moderate radiation. Radiation affects generally the most sensitive functions first because, as we have stated above in analysing the effect of the radiation, the structure and activities of the organisms are found to be the more important component of the reaction, in contradistinction to the radiation, which represents a more or less non-specific interference with the functions of the cell.

In this discussion of the quantitatively graded effects of radiation, it might be of interest to refer to some recent experiments of Auer and Witherbee, who showed that the destructive effect of Roentgen rays on the skin of the rabbit can be much

weakened by sensitizing the animals to horse serum through repeated injections of this substance, about two weeks preceding the radiation of the rabbits. These authors assume that the formation of sessile anaphylactic antibodies in various tissues of the animals changes the tissues in such a way that they become more resistant to the action of radiation. All these observations point to the conclusion that the condition of the system on which the rays act is of primary importance in determining the end result of the interaction between rays and organisms.

In recent years this interaction has been elucidated from still another point of view. Various investigators have concurrently found a definite quantitative relation between intensity of radiation or time of exposure and the biological effects of radiation. Thus Congdon concludes that the degree of retardation in the regeneration of tubularia under the influence of beta rays increases slowly with lengthening exposure, but the degree of retardation relative to the length of exposure decreases with lengthening exposure. According to Chambers and Russ, the destruction of bacteria under the influence of radium rays follows a logarithmic curve, if the number of colonies is represented on the ordinates against the time of exposure as abscissae. Davey measured the length of life of the beetle Tribolium confusum, after exposure to x-rays. He concludes that there is a definite mathematical relation between the duration of life of these organisms and the logarithm of the total x-ray dose. According to the recent investigations of Redfield and Bright, who measured the effect of radiation with beta rays on the eggs of Nereis by the resulting increase in the thickness of the egg membrane, the volume of membrane varies directly with the logarithm of the time of exposure. Davey, as well as Redfield and Bright, called attention to the similarity of this mathematical relation with the Weber-Fechner psycho-physical law. In both cases the effect varies in direct proportion to the changes in the quantity of physical stimuli and in inverse proportion to the strength of the stimuli. In one case we have to deal with a biological reaction, in the other case with a psychical reaction.

We notice furthermore that this quantitative law holds good independently of the organism used as a test object, and it applies to x-rays as well as to beta rays. This is a further indication of the essential similarity in the action of various kinds of radiation on living things. We may even go a step further and conclude that not only x-rays and alpha, beta, and gamma rays act essentially in a similar manner on living organisms and differ from each other mainly in the strength of their effects, but that even other radiations, as, for instance, the ultraviolet rays, act very much like Roentgen and radium rays.

In the case of the short waved gamma and Roentgen rays, as well as in the case of the longer waved ultraviolet rays and visible rays, those waves are biologically active which are retained in the cell; in all these cases the effects are proportionate to the amount of absorption. In the case of the ultraviolet rays Bovie could show that the longer waved rays and the shorter waved fluorite rays differ in their action on paramaecia in accordance with the part of the cell in which the rays are absorbed. The rays which are held back by the cytoplasm cause here vacuolization and immobilization, while nuclear division may still proceed; on the other hand, the rays which penetrate to the nucleus prevent cell multiplication. But essentially the action of ultraviolet rays on the developing eggs and on paramaecia is quite comparable with the effect of radiations given off by radium and with the effect of x-rays. As we have shown above, even the effect of graded intensities of heat on pieces of tumors is in many respects similar to the effects of radioactive substances and of x-rays.

In all these cases we have to deal with biological reactions of organisms in which the structure and composition of the organism is the important and specific component in the reaction, and the change in the physical environment represents merely a non-specific interference with the life processes of the organism, towards which the organism reacts in a way tending to reëstablish the old equilibrium. If the interference is relatively slight, a temporary intensification of the reaction of the stimulated system may result, otherwise an inhibition may follow, which

may be overcome, however, if the injury is limited in intensity. This conception does not necessitate the assumption that all these agencies produce exactly the same initial chemical changes in the cells. It is possible that the initial change in all these cases consists in a splitting off of an electron from atoms and in a concomitant ionization. Yet various radiations may produce changes of an entirely different character. In this connection we may, for instance, refer to the recent findings of Weigert relative to the action of polarized light on silver chloride compounds. In this case we have apparently to deal with effects other than those of ionization.

9. PHENOMENA OF IMMUNIZATION AFTER REPEATED RADIATION

We have mentioned in a previous paragraph that if radiation is applied to the skin repeatedly at suitable intervals, a cumulative effect may become noticeable (Werner). Usually, however, the opposite occurs; the radiated cells or the radiated organisms become more resistant to the effects of raying, if the radiation is repeated a number of times; and even if at first a stimulating effect should be noticeable, this usually decreases in the course of repeated radiations. Thus Russ, Chambers and Scott noticed, that if the number of lymphocytes has returned to normal after a radiation, these cells are refractory to the effects of a second radiation during the next twenty-four hours, but subsequently they acquire again their original responsiveness. The decrease in sensibility of nerve tissue which follows the application of radium is said to become less after several radiations. Similarly it has been observed by many investigators that in the treatment of cancer, leucaemia, or benign new formations like keloid, the first dose is the most effective one. In accordance with this statement is, for instance, the experience of Domenici, who finds that if radiation is applied to tumors at long intervals the effect becomes less and less. Even after repeated ineffective exposures to radium, tumors may become more resistant to an otherwise efficient dose (Ewing). It has been found that recurrences of tumors are usually refractory to radiation.

Whether this increased resistance is due to an increased resistance of the radiated cells (cell immunity) or to a reaction of the whole organism, has apparently not yet been determined. Transplantation experiments could perhaps decide between these two possibilities. We hope to be able to attempt some determinations of this kind. In the case of an acquired immunity against the effects of colloidal metals, which can be demonstrated in animal tumors, this immunity must in all probability be referred to an active immunization of the radiated cells. In this case the acquired immunity is demonstrable even in cells transplanted into a new host (Fleisher and Loeb). At present it seems more probable that the immunity is a cellular one. Thus in leucaemia repeated radiation calls forth the production of new tissue, consisting of large mononuclear cells, especially in bone marrow and retroperitoneal lymph nodes, and these newly formed cells seem to be more resistant to the effects of radiation (Warthin). It is probable that in other cases, too, the increased resistance may be attributed to newly formed cells. In a similar way, according to Werner, repeated exposures of epidermis to a low temperature call forth the formation of generations of new cells, and in the end a layer of epithelium is produced which is more resistant to various kinds of injurious influences than were the original cells.

10. INDIRECT EFFECTS OF RADIATION ON RESISTANCE AND IMMUNITY

We have referred to the great sensitiveness of lymphocytes to the effects of radiation, and also to the fact that it is possible not only to decrease but also to increase the number of lymphocytes in the mouse (Murphy). According to Murphy some important secondary changes are called forth as a result of these experimentally produced variations in the number of lymphocytes. In order to appreciate the significance of these changes, it is necessary to consider very briefly the activity of lymphocytes, as well as of blood vessels and connective tissue cells in the organism, whenever they come in contact with new tissue or tumor. In association with Myer, Hesselberg, Sale, Kerwin, Seelig, we have shown that, in the guinea-pig, autotransplantation of

tissue is followed by an early response on the part of the vascular endothelia of the host which readily grow into the tissue. The ingrowth of fibroblasts is likewise such that a normal cellular tissue is formed, sufficient to guarantee the production of a satisfactory stroma. Lymphocytes, on the other hand, are not attracted. In case of homoiotransplantation, the strange tissue calls forth a more limited reaction on the part of the vascular endothelia, while the fibroblasts of the host, on the contrary, are very strongly attracted; they grow around or into the strange transplant and form dense fibrous tissue, which may strangle and thus injure the transplant. Some connective tissue cells may actually invade the transplanted parenchyma. Lymphocytes are attracted in large masses and help to destroy the tissue. In syngenesiotransplantation (exchange of tissue between relatives) the results are in certain cases intermediate between those of auto- and homoiotransplantation. Connective tissue and vascular endothelium may behave as in autotransplantation, but lymphocytes later tend to destroy the transplant. In heterotransplantation the transplanted tissue is directly injured through the body fluids of the host. The fibroblasts of the host form a wall around the strange tissue and produce a capsule of dense fibrous tissue which may be infiltrated with large masses of lymphocytes. They behave towards the strange tissue in a way similar to an irritating foreign body.

We see then that fibroblasts and lymphocytes represent instruments of defense against tissue which is not quite compatible with the cells of the host, but more or less strange, and that there is a relationship between the character and intensity of the reaction and the incompatibility of the tissue. Parallel with these researches, various investigators (Burgess and Tyzzer, DaFano, Rous and Murphy and others) have shown that lymphocytes play a significant rôle in the immunization against a tumor which originated in another animal. The lymphocytes collect around and injure the transplanted tumor tissue; the connective tissue around it becomes densely fibrous. After autotransplantation of tumors these reactions are lacking. In the case of tumors these phenomena have usually been interpreted as due to im-

mune reactions which occur in the organism into which the strange tumor has been introduced; but we believe it more probable that they are primarily direct reactions of the host cells against the introduction of strange tissues of a homoio or syngenesio character, and that they are of the same nature as the reaction seen after transplantation of normal tissue. Only secondarily reactions of immunity may be added to these primary reactions. While after autotransplantation these reactions are usually absent, somewhat similar reactions may occur in case a spontaneous tumor retrogresses in toto or in part.

Now the investigations of Murphy seem to show that it is possible to increase experimentally the reactions of the host against strange (homoio) tumor tissue or even against an auto-transplant, by all those means which stimulate the activities of the lymphocytes such as an appropriate dose of soft x-rays. A decrease of lymphocytes, on the other hand, is followed by a decrease in the resistance against a tumor transplant, and also against a piece of normal tissue of a different species. However, Murphy and his collaborators have not shown so far that by such means the growth of a spontaneous tumor (auto-tumor) can be influenced. Of particular interest is also the statement of Murphy that tissues which have been directly exposed to soft x-rays form an unfavorable soil for the growth of (homoio) tumors and that in this case lymphocytes collect around the transplant.

While these conclusions of Murphy have been controverted by Prime and others, they have been confirmed by Mottram and Russ, as far as these authors carried out analogous experiments, and it seems very probable that in the main Murphy's conclusions as to the effects of an increase and decrease of lymphocytes on the immunity against tumor growth are correct. According to Murphy the significance of lymphocytes in the immunity against tuberculosis is of a nature similar to their effect in tumor immunity; but this view also has been controverted. Whatever the effect of stimulation or destruction of lymphocytes on immunity in tuberculosis may be, we may conclude that x-rays may indirectly influence the defensive reactions of the host against strange tissues and tumors.

We shall briefly refer to an apparently related indirect effect of radiation. If animals are exposed to the action of x-rays at an early stage of immunization against red blood corpuscles, the production of antibodies is very unfavorably affected. Hektoen interprets this result as secondary to an injurious action of the rays upon lymphatic organs and bone marrow, probably the principal seat of antibody production. At a subsequent period following radiation, however, antibody production may, on the contrary, be influenced in a beneficial way through a previous radiation. Quite recently Hektoen and Corper noticed an unfavorable effect on the formation of antibodies following the intravenous injection of an active deposit of radium emanation. As Bagg had previously shown, and as these authors confirm, the emanation thus introduced into the organism acts injuriously on various organs like liver, suprarenals, lungs, but in particular it injures the blood forming or blood destroying organs, bone marrow, spleen, and lymph nodes. It furthermore reduces the number of red corpuscles and leucocytes, especially of lymphocytes, in the circulating blood. According to Cluzet and Chevallier, this effect may be temporarily preceded by an increase in erythrocytes and polymorphonuclear leucocytes. It is very probable that there exists a parallelism between the action of x-rays and the emanation on the blood forming organs, on the one hand, and on the formation of immune bodies on the other.

11. TOXEMIA AFTER RADIATION

Under various conditions a toxic condition is found in animals subjected to the effects of Roentgen rays. Thus Contamin noticed that if in a mouse a large tumor was absorbed as a result of radiation, the animal usually died on the fifth or sixth day following radiation. On the other hand, according to Ewing, large tumors can be absorbed without intoxication and this author suggests that bacterial action may be implicated in the effects following destruction of large parts of the tumor. We have already referred to the findings of Neuberg, according to whom autolytic ferments may be stimulated to increased activity by a preceding radiation, while other protein splitting or coagulating

ferments are inactivated. Edsall and Pemberton accordingly attributed the toxemia noticed after radiation to an increase in autolytic processes in tissues generally. A similar view has been expressed by Regaud, who cites as proof of the far reaching effects of radiation the myocarditic changes which he observed in radiated animals. The nitrogenous constituents of a non-protein nature are increased in the urine and in the blood during the state of toxemia in radiated dogs (Hall and Whipple), a finding which would be compatible with the assumption of an increased destruction of tissues. Joltram and Benard, on the other hand, attribute the toxemic condition to destructive processes which take place in the blood as the result of radiation. They not only find a diminution in the white blood cells, but also a diminution of the blood proteins, in contradistinction to Hall and Whipple, who seek the cause of this condition in an intoxication of intestinal origin, to which they refer the accompanying diarrhea and vomiting; they find a diminution in size and a hardening of the spleen, in addition to the changes in the composition of the blood and urine to which we referred above, and they compare the toxemic symptoms following radiation to the intoxication following injection of albumoses or intestinal obstruction. However, while in the intoxication due to proteoses, the coagulability of the blood is diminished, in the radiation toxemia it is, on the contrary, increased, according to the concurrent results of numerous investigators. This renders the interpretation of Hall and Whipple doubtful. On the other hand, the view of Joltram and Benard, who consider changes in the blood as responsible for the intoxication, does not seem to be very well founded either, because a marked diminution of white blood cells may be brought about without any accompanying symptoms of intoxication. At present the view that a breakdown and solution of protein substances in the radiated tissues and the subsequent diffusion of these substances into the circulation is the cause of the intoxication, is the most probable interpretation. The recent experiments of M. Giraud, G. Giraud and Parès support very strongly this view. They found that radiation of the spleen is followed by intoxication, but that this effect can be prevented

as long as the vessels of the spleen are clamped. As soon as the substances which have been produced under the influence of radiation are allowed to enter the circulation, the general effects of radiation become noticeable and in particular the changes in the blood, consisting in a decrease in the number of leucocytes and in a shortening of the coagulation time of the blood. In accordance with this interpretation is, furthermore, the observation that the intoxication is the more severe, the greater the skin area which is exposed to radiation and that it may, for instance, be absent after vaginal radiation (Friedrich). In addition to the size of the radiated area, the character of the organ which is radiated seems to be of importance. Radiation of organs in which solution processes of proteids occur more readily, is followed by more severe consequences than radiation of more resistant tissues.

12. A COMPARISON OF THE EFFECTS OF RADIATING TISSUES IN VITRO AND IN THE LIVING ORGANISM

It is a question of great practical importance to determine the dose of radiation necessary to cause the destruction of a cancer in the living organism. It would therefore be of advantage, if it were possible, to standardize this dose in experiments in which pieces of tumors are kept in vitro and exposed to radiation of variable intensity under conditions which can be more easily controlled than those existing in the living body. Thus the differences in the behavior of different kinds of tumors could be determined accurately and without the disturbing effect of complicating factors which exist in the living organism. Such determinations have been carried out on a large scale, especially by Wood and Prime. In part we have referred to these investigations above. These authors conclude that in order to kill small pieces of tumors in vitro two to eight erythema doses are required, that both sarcomata and carcinomata vary individually in resistance, and that the lethal dose required in the living organism is about 20 per cent larger than that required to kill the same kind of tissue in vitro.

The experiments of Wedd and Russ, Prime, and Kimura have indeed shown that there exists a certain parallelism in the effects of the rays on the piece of tumor radiated in vitro and its subsequent behavior after transplantation in a living animal. The greater the reduction of the mitoses in a piece radiated in vitro, the less the growth energy of the tumor which develops after transplantation of the radiated pieces. This fact does not, however, prove that radiation of a piece of transplanted tumor in vitro and its growth after transplantation into another individual of the same species, corresponds in every respect to the effect of radiation of a spontaneous (auto) tumor in the living organism.

While in certain respects the effects of radiation in vitro seem to be identical with those obtained after radiation in the living organism, in other respects some differences seem to exist. (a) The effect of radiation on tissue in vitro as well as in vivo is primarily a direct one. Radiation in both cases affects the tumor cells, which are injured or stimulated according to the quantity of rays received; the primary effect does not consist in a stimulation of the surrounding connective tissue, as has been maintained by several writers; the tumor cells are much more accessible to the direct effects of radiation than the tissues surrounding the tumor. (b) Radiation of tumor in the living organism, especially of the peripheral parts of the tumor, which are most important from a practical point of view, affects a tissue living under optimal conditions as far as temperature, supply of oxygen and other food material, and elimination of waste products are concerned. In such a tumor the mitotic proliferation is maximal. Radiation in vitro is, in all probability, carried out under less favorable conditions. While the temperature coefficient of radiation is only slightly above one and the direct effect of the differences in temperature on radiation in vitro near the freezing point and in vivo at body temperature can therefore not be very considerable, still in an indirect manner the difference in the temperature prevailing in vitro and in vivo may play a certain rôle.

The low temperature of the pieces kept in vitro as well as the relative lack of oxygen under these conditions may lead to a

considerable reduction in the mitotic activity of the tumor cells; and inasmuch as the effect of radiation is lessened, if it affects less actively proliferating cells this factor would tend somewhat to decrease the effectiveness of radiation in vitro. (c) Radiation in vitro excludes to a great extent the effects of absorption and of scattering of the Roentgen rays. The former is quantitatively of much less significance than the latter, if very penetrating rays are used. While the average amount of scattering in a unit of space increases with increasing wave length of the rays, and the absolute amount of scattered rays might therefore be greater, if soft rays are used, the important factor to determine is the relative amount of scattered rays, as expressed in the proportion between scattered and direct rays. This relative amount of scattered rays is the greater, the deeper the radiated area, the larger the surface of the radiated organism, the more centrally situated the tumor in the radiated area, and the more penetrating the rays. Under certain conditions the scattered rays may equal twice or even three to four times the amount of direct rays. The scattered rays therefore play an infinitely greater part in the living organism than in vitro, and they are presumably of much greater significance under conditions of radiation such as obtain in man, than they are in the mouse or rat. If the direction in which the rays penetrate the organism is unfavorable, certain parts of the tumor may receive, instead of direct rays, merely an insufficient amount of scattered rays and thus, instead of a destructive dose, a stimulating dose may be given to the cancerous tissue. (d) Secondary gamma and beta rays may be given off by the tissue directly surrounding the tumor and the amount of these rays may differ in vitro and in the organism; however, this factor is presumably of little importance. (e) Reactions on the part of the host tissue may play a certain rôle. While it is almost certain that the surrounding connective tissue and the lymphocytes as a general rule are incapable of destroying spontaneous (auto) tumors to any considerable degree and are therefore as a means of defense against the growth of natural tumors presumably of no great practical importance, it may perhaps be different in the case of tumors which have previously been injured in their vitality and

possibly modified in some way through previous radiation. In this case it is not impossible that the invasion of the weakened tumor by connective tissue cells and its encapsulation by fibrous tissue may become significant. We have previously seen that tumors injured by heat may be surrounded by dense fibrous tissue, and while tumor cells which possess a normal growth energy may not be seriously impeded by such an obstacle, tumors experimentally weakened through heat or radiation may not be able to break through such a barrier. As our experiments on the effects of heat, and the more recent experiments of Wood and others on the effects of radiation have shown, all degrees of diminution in growth energy may be obtained by such means. In certain cases the inhibition thus produced may perhaps last longer than the life of the individual in which the tumor developed; in other cases the inhibition may be only temporary. Furthermore, we have seen that heated, as well as radiated, tumors may in the end regain their full growth energy, especially after repeated transplantation, although this may even occur without transplantation. Transplantation acts in this case as a growth stimulus which would not come into play if we have to deal with spontaneous tumors in man.

Generally, the tumors used for radiation in vitro are transplanted tumors of a homoio-character; as such they call forth defensive reactions on the part of the host following transplantation. In the case of tissues and tumors of auto character, the defensive reactions on the part of the host tissue are lacking as far as the action of lymphocytes is concerned. As we have seen, under normal conditions auto tissues and auto tumors do not attract lymphocytes to any considerable extent. In this respect radiation in vitro and radiation in man differ. There remains however the possibility that in contradistinction to normal tissues and tumors of an auto character, in auto tissues or auto tumors which have previously been injured through radiation lymphocytes do appear around the injured tissue or that they may otherwise exert an influence on these tumors.

Now according to Murphy, increase of lymphocytes experimentally produced through x-rays or otherwise, increases the

power of resistance of the host tissue, not only against transplanted homoio-tumors but even against pieces of spontaneous tumors after retransplantation into the same animal in which they took their origin. This would imply an effect of lymphocytes on auto tissues which is entirely lacking under usual conditions. Yet, even if we should accept the conclusion that such an effect on auto tumors does exist, it would still remain very doubtful if it would apply to spontaneous tumors in man for two reasons: In the first place, the dose of radiation used in man must be sufficient to destroy or to injure the tumor cells. Thus a dose of rays would be required which would not only not increase, but on the contrary, decrease the number of circulating lymphocytes. It would therefore rather tend to weaken the power of resistance of the host against the tumor growth. In the second place, Murphy noticed an influence of radiation only on transplanted pieces of auto tumor, not on the original spontaneous tumor. Now it is a well established fact that tumor tissue is much more accessible to defensive reactions on the part of the host tissue during the first period following transplantation, at a time when the tissue is not yet thoroughly established in the new environment, than during later periods when the vascularization is complete. Conditions which can suppress the growth of a tumor in the first period can no longer do so, once the tumor has been fully established; and the spontaneous tumors, with which we have to deal in man, are fully established tumors.

As we have mentioned above, Murphy has recently shown that coincident with an increased resistance to the growth of homoio-tumors, transplanted into tissue radiated previous to the transplantation, there develops an increased local lymphocytic reaction. These experiments show that the tissues of the host directly subjected to radiation are an unfavorable soil for the development of homoio-tumors during the first period of their growth; it is improbable that these results apply to the radiation of well established auto tumors.

In addition to the local reactions of the host tissues to the radiated implanted tumor, there may come into play distant actions of which, however, relatively very little is known at the

present time. But certain instances of such distant action have been established recently. Thus we have referred above to the experiments of Murphy, who showed that the stimulating action of soft x-rays on the lymphocytic organs depends upon the production of a substance circulating in the body fluids; and in a similar way M. and G. Giraud and Parès found that the destruction of leucocytes under the influence of radiation depends on the production of leucolytic substances which enter the circulation.

We may then conclude that: (a) the main effect of radiation on tumors consists in a direct injurious effect of the rays on the tumor cells. (b) That secondarily host reactions may play a part in holding in check for variable periods of time, or in injuring still further, tumor cells which have been primarily injured by radiation; that, however, results of experiments in which the number of lymphocytes was artificially increased, and an effect obtained in the case of transplanted tumors cannot, as yet, be directly applied to spontaneous tumors in man. (c) That radiation in vitro does not in every respect reproduce the conditions prevailing during radiation in the living organism, inasmuch as the physical factors of radiation prevailing in vitro and in vivo differ and inasmuch as the state of the tissues is not the same during radiation in vitro and in vivo; furthermore we have referred to the fact that the host contributes to the effects of radiation through contiguous tissue reactions as well as through distant action. Yet, while making due allowance for these complications, there seems to be after all a certain parallelism between the results of the radiation of tumors in vitro and in vivo, as has been shown especially by Wood and Prime.

The recent experiments of Friedrich, who found that, if conditions of radiation are chosen in such a way that an unfavorable general influence on the health of the patient, and especially a radiation cachexia, are avoided, the results of radiation on the tumor are improved, likewise indicate that secondary reactions on the part of the host tissue play a part in the effect of radiation on the tumor.

In this connection we may refer to some recent experiments, in which the attempt has been made to obtain an increase in the

effects of radiation on living cells by combining successfully the action of heat or other physical or chemical agencies and of radiation. A number of earlier investigators had already attempted by these means a sensitization of tissues to radiation. Recently Bovie has shown that after a preceding radiation albumin is more readily coagulated by heat. This author further found that by exposing paramaecium to fluorite rays, the animals are subsequently sensitized to heat effects. By increasing the temperature after raying, the latent period of the rays is shortened and the effect is the greater the higher the temperature used. The temperature in these experiments was, however, not sufficiently high to affect normal organisms. Rohdenburg and Prime combined heat and radiation in the treatment of tumors. They exposed the tumor pieces to a temperature of 40°. Neither the dose of x-rays used nor the heat alone were lethal, but both combined killed the tumor, if exposed in vitro. The results were independent of the order in which heat and radiation were combined. While it still remains to be determined how far these results are applicable to the treatment of tumors in the living animal, from a theoretical point of view these experiments are interesting. They show that just as a summation is possible of the effects of a natural weakness of certain cells and of injury experimentally produced through the rays, so it is also possible to cause a summation of the effects of two experimentally produced injuries, such as those of heat and radiation.

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A CRITIQUE OF TUMOR RESISTANCE

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An animal in which a transplanted tumor has receded is often resistant to a second inoculation. When this was first noted it appeared to be an observation of the greatest importance, and because it was not possible to have always on hand a number of such animals sufficient for a thorough investigation of the phenomenon, experimental pathologists set themselves the task of finding some other way to elicit resistance. It was soon discovered that previous treatment with a normal tissue of the same species is efficacious in producing resistance to some transplantable tumors, though not to all.

Now although it was proved not long afterward that an animal cannot be made refractory to inoculation of a graft from its own tumor (1) the clinician continued to cherish the hope that some practical use might be made of resistance, and persisted in attempting to cure neoplasms in man with various preparations of normal tissues. Worse still, emulsions of viable autologous cancer cells were sometimes introduced, with the unhappy result that in certain cases these cells survived inoculation and gave rise to new tumors.

It was proved, too, that an animal cannot be made resistant to tumor inoculation by treatment with spleen or other tissues from his own body. This rendered hopeless any attempt to elicit the refractory state in a cancer patient by way of his own lymphocytes, since there was now a double reason for failure: The fact that an animal cannot be made resistant against a tumor that has originated within its own body, and the fact that no resistance can be elicited with an animal's own tissues. Thus closely are the lines drawn in cellular immunity!

So there is not, and there never has been, the slightest reason to anticipate a cure by the production of antibodies, a method that has yielded such brilliant results against the bacteria; for the difference is precisely the difference between fighting a traitor within the body, and repelling an invader from without.

A somewhat similar distinction can be drawn between the spontaneous tumor, composed of cells native to the animal's own organism, and the transplanted new growth, made up of the cells of one animal proliferating as best they can in the body of another—that is to say, under conditions more or less unfavorable according to the adaptability of the tumor. The difference between the two is well shown by the comparative frequency with which they regress. While 50 or 75 per cent of the growths in certain transplanted strains may recede, there have been but twelve instances of regression among some two thousand spontaneous mouse carcinomata that have been under observation at the Crocker Institute during the past ten years. In the case of man, the disappearance of a malignant new growth is even more rare. Rohdenburg (2), who collected most of the recorded cases a few years ago, could find only 302, and a considerable number of these he regarded as open to suspicion. Indeed, one of the patients, until then apparently in good health, died of a rapid recurrence almost before the paper was off the press. It would perhaps be safe to say that there have been hardly more than 200 permanent regressions in man in the past fifty years, a group so small in comparison with the total number of persons that have died of cancer during this period as to be practically negligible.

Now while by appropriate measures an animal can be made resistant to the implantation of certain tumors, these methods have not the slightest effect upon an established neoplasm; that is, they prevent the entrance of capillaries and fibroblasts into a new graft, but do not affect a tumor already provided with blood-vessels and stroma. If resistance is thus powerless against a transplanted neoplasm, leading what must be in many cases a sort of hand-to-mouth existence on a foreign soil, there is no reason to expect it to be successful against a spontaneous one,

which is so much more favorably situated. Yet the attempt has been made over and over again, and sometimes to the great detriment of the patient, as has already been indicated.

It may be that the pathologist was partly to blame, for he continued loosely to refer to resistance as "immunity," thereby leading any one not entirely familiar with the new science of immunology to the natural conclusion that the conquests of bacteriology were about to be repeated in another field. But, always less sanguine than the clinician, he was not surprised to see the earlier and momentary hope of success immediately crushed by failure. For not only has resistance been disappointing in a clinical sense, but it is full of what can only be called, in our present ignorance, actual contradictions, and of problems which have proved so far utterly insoluble.

Thus, it is not yet known whether the resistance which characterizes an animal with a receding transplanted tumor is the cause or the outcome of regression. It has been said, it is true, that resistance precedes spontaneous cure (3). Still, recession must begin some time before it becomes evident in the tumor, so that the precise moment at which it sets in cannot really be determined.

The four most striking problems of the refractory state may now be passed in brief review.

First, it is by no means possible to induce resistance against all transplantable tumor strains. The sarcomata are a notable example, most of which resist all efforts to make animals insusceptible to them; and occasionally even a carcinoma is encountered against which resistance cannot be elicited. Furthermore, every animal treated is not always made insusceptible, even to a tumor strain against which it is easy in general to produce resistance.

Second, why is the injection of normal homologous tissue, so potent against a new graft, entirely without effect upon the growth of an established tumor? Certainly not because an animal already bearing one cannot be made resistant, for Russell (4) has shown that tumor-bearing animals can be made refractory to a second inoculation.

In the third place, a receding tumor, like any growing one, is made up of a necrotic central portion and a marginal zone of healthy cells. If it be a general reaction on the part of the host that brings about the disappearance of a neoplasm, why are these marginal cells the last to die? Not only do they come into direct contact with the tissues of the host, and therefore with any hypothetical deleterious agent resident therein, but they are the only cells of the tumor with an adequate circulation, so that they are most exposed to any harmful substance, if such there be, in the blood stream. Whether one is disposed to regard the refractory condition as a tissue reaction or as a blood reaction, the difficulty of explaining the longer survival of the periphery in a disappearing tumor is equally embarrassing.

Fourth, it not rarely happens that a tumor which has begun to regress recovers itself, and at the next weekly charting is found to be growing again. Not so frequently, yet often enough to be taken into account in any explanation of the resistant state, a neoplasm will begin to recede, will next pass through a phase of renewed growth energy, and then finally undergo complete regression. How can this be explained when insusceptibility is ascribed to a generalized change in the organism of the host? Indeed, it would seem actually hazardous to refer the disappearance of a tumor to constitutional changes in the animal bearing it, for not infrequently one new growth will remain stationary, or will even recede, while a second one in the same host is actively advancing.

Thus the doctrine of generalized resistance, when critically examined, is found to be inadequate. If regression were brought about by constitutional changes in the host, it should not be difficult to find , in addition to the recession, other proof of injury to the tumor as a whole. But the disappearing tumor does not appear to be uniformly damaged, for it is common enough to encounter nodules of firm, translucent, and apparently healthy tissue, alternating with degenerated and cystic portions. Or again, a tumor so badly damaged throughout as to be incapable of further growth, might be expected to have fewer mitotic figures than an actively growing one. This has been said to be the case

(5), but it cannot be the general rule, for other observers have found them to be not noticeably reduced in number (6).

It is well known, of course, that the number of division figures is not an infallible index of growth speed, for the most slowly proliferating tumors in man may contain a large number, as McConnell (7) has shown. But there is all the difference in the world between a tumor that is growing, no matter how slowly, and one that is receding; and one would hardly expect to find the cells of a dying tumor dividing as vigorously as those of a growing one. Yet the examination of a large number of disappearing tumors by the present writer showed them often to contain as many mitotic figures as are found in an actively growing one (plates 1 and 2). The fields from which these plates were photographed were chosen at random and represent, not the exception, but the rule.

If the receding tumor does not depart from the growing one in respect to the number of its mitotic figures, neither does it differ in any other discoverable morphological feature (plate 3); for it is quite impossible to distinguish under the microscope between a growing and a regressing neoplasm, unless the latter be entirely necrotic. Furthermore, division figures can be found up to the very edge where healthy tumor adjoins necrosis, and it is difficult to escape the conclusion that the peripheral cells continue in active proliferation until the moment when they are overtaken by death. Indeed, it seems almost as though there were two waves sweeping over a tumor—one of growth, and a pursuing one of death; and that if the latter overtake the former the tumor undergoes regression.

The vigorous division going on in those cells that have not yet perished makes it hard to believe that regression is due to any deleterious action by the host against the tumor as a whole, because it is at the periphery of the neoplasm that these surviving cells are found. And equally inadequate, in the face of these abundant division figures, seems any hypothesis referring spontaneous cure to some change in the biology of the tumor cells themselves, by virtue of which they become unable to proliferate further. Why should such a change affect always the cells at the

centre of the growth? Nevertheless its possibility cannot be entirely dismissed.

It may be objected that the mitotic figures in a receding new growth are merely agonal, but if this were true regressing neoplasms would not be transplantable. Yet they are, as White and Loeb (8) have shown. Bashford, Murray, and Bowen (9) too, in discussing the low transplantability of retrogressing neoplasms, mention specifically such a tumor that gave rise to one tumor in nine mice. No doubt the number of daughter tumors obtained from a disappearing neoplasm is small, and proliferation not so energetic as it is in those descended from a vigorously growing one; both groups of authors agree that this is the case. The wonder is that there should be any growth at all.

EXPERIMENTAL

Partly because of such reports and partly, too, because of the emphasis constantly laid upon the necessity of selecting for transplantation none but the most vigorously growing tumors, the extraordinary fact that regressing neoplasms are transplantable has been lost to view. A small yield of daughter tumors without much proliferative activity might reasonably be expected, because many of the fragments inoculated from a receding new growth must be composed of dead or dying cells.

On account of the importance of this question and the small number of observations to be found in the literature, it has been taken up anew by the present writer. Several receding Jensen rat sarcomas were inoculated, with uniformly negative outcome; but though these were transplanted as soon as they were discovered to be regressing, microscopic examination showed almost complete necrosis in some, and total necrosis in the rest. Other neoplasms, however, which recede more slowly and retain a margin of healthy tissue after the chart-book shows that regression has set in, can be transplanted with a fair degree of success (figs. 1 to 5).

Because of the variations in growth energy shown by any one tumor strain, it was a matter of considerable difficulty to select controls for these experiments, for no tumor has a standard
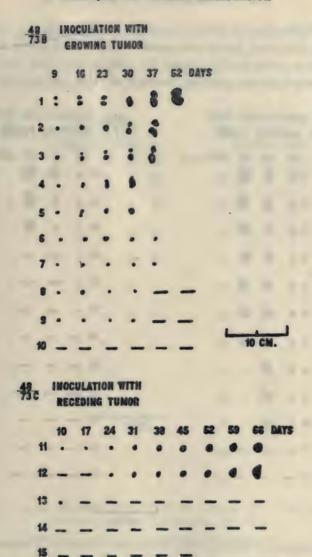
FIG. 1. COMPARISON BETWEEN THE DAUGHTER TUMORS OF A GROWING AND A
RECEDING MOUSE CARCINOMA, No. 48

The same breed of mice was used for both series

and inflexible growth rate. In order to eliminate any chance of personal bias and to obtain control series that could be fairly used, there were finally chosen for comparison five stock series transplanted from growing tumors of the generations which
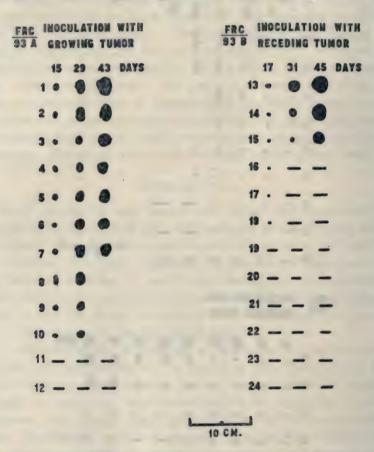
FIG. 2. COMPARISON BETWEEN THE DAUGHTER TUMORS OF A GROWING AND A RECEDING FLEXNER-JOBLING RAT CARCINOMA

The same breed of rats was used for both series

contained the receding ones, inoculated into the same breed of animals, and as near as possible to the date when the regressing neoplasms were implanted.

These experiments agree entirely with those just cited; proliferation is not so active as in the case of daughter tumors transplanted from vigorously growing neoplasms, and the number of successful inoculations is small. Nevertheless, the growth of

FIG. 3. COMPARISON BETWEEN THE DAUGHTER TUMORS OF A GROWING AND A RECEDING MOUSE CARCINOMA, No. 48

The same breed of mice was used for both series

these descendants of regressing tumors is better than might have been expected and, in fact, better than is sometimes seen after implantation of an actively growing neoplasm.

In brief, then, the surviving cells of a receding new growth undergo vigorous mitosis, and can be successfully propagated,

FIG. 4. COMPARISON BETWEEN THE DAUGHTER TUMORS OF A GROWING AND A RECEDING MOUSE CARCINOMA, No. 11

The same breed of mice was used for both series

more or less in proportion to the amount of surviving parenchyma that this contains. They act after transplantation like cells that have been injured by heat or by radiation previous to inoculation; in other words, no peculiar, specific damage appears to have been inflicted upon them.

IRS 19 21 B	INOCULATION WITH GROWING TUMOR					
	14	21	28	42		
1	•	●	◕	•		
2	●	●	◖	●		
3	●	●	◕	◕		
4	•	●	●	•		
5	●	●	•			
6	●	●	•	•		
7	●	●	•	—		
8	●	●				
9	●					

10 CM.

IRS 19 22 A	INOCULATION WITH RECEDING TUMOR					
	14	21	28	35	42	49 DAYS
10	•	•	●	●	●	●
11	•	●	●	●	●	●
12	—	—	—	—	—	—
13	—	—	—	—	—	—
14	—	—	—	—	—	—
15	—	—	—	—	—	—
16	—	—	—	—	—	—
17	—	—	—	—	—	—
18	—	—	—	—	—	—
19	—	—	—	—	—	—
20	—	—				
21	—	—				

FIG. 5. COMPARISON BETWEEN THE DAUGHTER TUMORS OF A GROWING AND A RECEDING RAT SARCOMA, IRS 19

The same breed of rats was used for both series

DISCUSSION

If the surviving cells in a receding tumor divide actively, and can be transplanted, it is hard to see wherein they differ biologically from those of a growing neoplasm. And if neither a general hostile reaction on the part of the host, nor the tumor cell

itself, can be proved responsible for spontaneous cure, it would perhaps be wise to shift our point of view, and search in some other direction for an explanation of this occurrence.

The blood-vessels have not yet been so thoroughly investigated as might be wished, and the remainder of this paper will be devoted to a discussion of these channels.

The general inefficiency of the circulation in neoplasms, and the frequent closure of vascular channels by thrombosis, have been so often described, as, for example, by Ribbert (10), that they have become proverbial. This thrombosis may be secondary to the extensive necrosis which so commonly affects malignant tumors of all varieties, and which has been ascribed to their vigorous growth and the consequent failure of their blood supply to keep pace with the incessant demands of a rapidly increasing parenchyma. Yet the English mouse carcinoma T, one of the most slowly growing of transplantable neoplasms, is, at the same time, one of the most necrotic.

Another cause for the necrosis may therefore be sought, and it will be worth while to regard the thrombosis for the moment as primary and the necrosis as secondary to it, if for no other reason than to see whither such a suggestion will lead.

It has been known for nearly a hundred years, of course, that occasional tumors disappear (11) when their circulation is totally interrupted, as by torsion of a pedicle or by thrombotic closure of an indispensable blood channel. The purpose of this paper is to inquire whether the latter process, though in a less sudden and dramatic form, may not underlie other examples of spontaneous cure.

The question is not entirely unreasonable, for the inefficient circulation of a neoplasm might well cause the accumulation of an excess of carbon dioxide in its blood-vessels; and this is known to increase the viscosity of the blood (12), while at the same time it dilates the capillaries (13), thus slowing the current and still further favoring thrombosis.

Again, it is believed (14) that thrombosis may accompany metabolic derangements in a surrounding tissue so slight as to escape the microscope; and if there is any one tissue in which

disorders of metabolism might reasonably be expected, it is the neoplasm.

A more concrete explanation for the thrombosis to which new growths are liable is the mechanical pressure which the rapidly increasing parenchyma must often exert upon its vessels, with all the possibilities for injury to their walls which this would entail; and a combination of the two factors just suggested, chemical and mechanical, in a tissue rich in coagulative substances (15), might be sufficient to bring about primary thrombosis.

The suggestion that thrombosis may be primary and necrosis secondary, is presented with considerable trepidation because it is difficult or even impossible to prove that thrombosis occurs first. In the older neoplasms so far examined, removed some twenty-five to thirty days after inoculation, there can be found thrombosed vessels surrounded by more or less healthy tumor cells, it is true (plate 4), and this would at first sight suggest that thrombosis is the primary lesion. But there is no way of eliminating the possibility that occlusion followed the extension of a thrombus, secondary to necrosis, further along the vessel. Examination of young tumors is not of much help, since advanced necrosis may affect the centre of a neoplasm as early as the tenth day.

Hence it is not desired to urge the proposal too strongly, but rather to present it for criticism and discussion; the more so because, while it does seem to clear up certain occurrences incompatible with the doctrine of resistance, it leaves others still without explanation.

Viewed from the suggested standpoint, the growth or the regression of a tumor becomes a question merely of the extent to which its vessels have been obliterated by thrombosis; and the difference between a growing and a receding neoplasm is a difference only in degree and not in kind.

Any resistance present in an animal with a receding tumor would then have to be regarded as incidental, rather than the determining cause of regression. So, also, the fibrosis so often described, and sometimes tentatively advanced as the cause of spontaneous cure, would be relegated to the position of a mere

replacement fibrosis; and it may be interjected here that many disappearing neoplasms show no trace of it, even though there be not a living tumor cell left (plate 3). Cellular infiltration and phagocytosis, no less than hemorrhage, would also have to be regarded as secondary occurrences.

The suggestion that thrombosis may be the primary cause of spontaneous cure elucidates a number of observations that cannot be otherwise explained. The continued growth of one tumor and the concomitant recession of another in the same host is a less puzzling phenomenon if we cease to regard spontaneous cure as the product of some generalized change in the constitution of the host, and invoke a local alteration in the tumor instead.

Again, the rhythms of growth energy that are seen from time to time in a single tumor, reaching their highest expression in actual cessation of growth and subsequent resumption, may be due to nothing more mysterious than circulatory disturbances following widespread thrombosis, and the later re-establishment of an adequate circulation. Though why all receding tumors are not rescued by a collateral circulation cannot for the present be known.

An explanation becomes possible for the fact that the chances of regression diminish, on the whole, as a tumor increases in size. For it seems reasonable to suppose that the longer a neoplasm remains in one host, the more intricate will become its vascular supply and the smaller the chance that this can be totally obliterated.

The suggestion under consideration may throw some light, perhaps, on the observation that transplanted sarcomata are more likely to recede than are transplanted carcinomata. In the sarcomata the vessels are in direct contact with the tumor cells, and it may be imagined that they are more exposed to injury of their walls by obtruding tumor cells than are the carcinomata, whose vessels run in a protecting stroma. Still, it is not desired to make too much of this point, for the Flexner-Jobling rat carcinoma, with a dense fibrous stroma, often recedes, whereas the Crocker Institute sarcoma 180 almost never does so, though its stroma is minimal in amount. Other factors un-

doubtedly enter here, if not elsewhere, and there is no desire to
insist that primary thrombosis is the sole cause of regression. In
the case of the chorioepithelioma, for example, it would be easy
to conclude that this neoplasm recedes so commonly because it is
the one tumor that preeminently attacks the blood-vessels; but
it must be remembered that this growth is not entirely autoch-
thonous, and that it may disappear because it is under less
advantageous conditions than one composed entirely of cells
native to the organism in which it has arisen.

No constant vascular changes in the vessels outside the
tumor have been observed. The paucity of small veins some-
times found about receding neoplasms (plate 5) has proved
not to be a characteristic feature, since these channels may be
quite as plentiful as they are around growing tumors.

A curious difference between some growing and regressing
tumors may be brought out by fixing the tumor in situ with
formalin after removing all hair from the flank, then cutting away
a large skin flap with the tumor, passing this through graded
alcohols, clearing it in several changes of benzol and, finally, in
methyl salicylate (synthetic oil of wintergreen). Growing tumors
thus treated tend to be of a pinkish hue, and with a lens it can
be seen that this color is due to the presence of innumerable small
blood-vessels; receding neoplasms on the other hand, are often
of a yellowish, translucent appearance, and contain no vessels,
or only a few large ones coursing over the surface. Whether
this difference is constant, and whether it is primary or secondary,
cannot yet be determined.

Turning now from the transplantable tumors of animals to the
spontaneous tumors of man, we can find a certain amount of
support for the suggestion under examination, though not all the
evidence is in its favor.

The retrogression of neoplasms after a sharp attack of fever,
that is to say after a temperature of from 104° to 105°F. has
continued for from forty-eight to ninety-six hours, assumes fresh
interest when it is remembered that it is just in fever that the
optimum conditions for thrombosis occur. The vascular endo-
thelium is easily injured by bacteria and their toxins, and the

most important cause of thrombosis is therefore present. Furthermore, the viscosity of the blood (16) and the amount of fibrinogen (17) are increased in such diseases as pneumonia and acute purulent conditions.

It is not impossible that the slower growth of malignant neoplasms in the aged is partly due to the increasing ease with which thrombosis occurs with advancing years (18).

Rohdenburg's observation (19) that a considerable proportion of the cases of spontaneous disappearance of a malignant neoplasm in man has occurred in connection with incomplete extirpation, is rather embarrassing and not easily to be explained, unless one wishes to assume that the operative manipulations were sufficient to cause complete thrombosis in those few fortunate instances where a cure supervened; or unless one is willing to adopt the equally feeble assumption that thrombosis set in because of the increased viscosity of the blood which is said to accompany narcosis (20).

In any case, enough has been said to indicate that even though primary thrombosis be a factor in the spontaneous cure of neoplasms, it may not be the only one.

TREATMENT

The suggestion which has now been outlined does not make the problem of treatment any more easily soluble, though it may help to explain what takes place in those rare instances where a transplanted tumor disappears after the administration of adrenalin (Bashford, Murray, and Cramer (21), Reicher (22), A. and H. Grünbaum (23) and others). Following the intense contraction of the arteries, the blood would be forced into the thin-walled vessels of the tumor; as these dilate, the current would be slowed, and opportunity given for stagnation thrombosis.

Assuming that the suggestion now under discussion is correct, the problem of treatment would be to find some agent capable of thrombosing the vessels of a tumor, and no others. The only reason for regarding such a discovery as anything but utterly chimerical is that the blood-vessels of many tumors are more

subject to thrombosis than are those of normal tissues, and that tumors do, after all, occasionally regress. But even though all the vessels of a tumor could be thrombosed, there would often remain single cells or small groups of cells, invading the surrounding tissue and supported, not by the blood-vessels of the neoplasm from which they escaped, but by fluids imbibed from the normal tissues about them.

A few experiments on mouse carcinomata have been completed, in which an effort was made to induce stagnation thrombosis, but the results were entirely negative so far as any control of tumor growth is concerned. It is true that the tumors were much softer than those in untreated controls, and that the microscope showed advanced edema and more widespread necrosis than is generally seen. But the growing margin was still healthy and its capillaries unaffected by thrombosis. And, still better testimony to the inadequacy of the treatment, the tumors continued their growth unchecked. Nor had the introduction of various substances credited with the power of favoring coagulation, in the hope of bringing about a differential thrombosis, the slightest effect upon any transplanted neoplasm.

If transplanted tumors cannot be influenced in their growth, it would obviously be idle to anticipate any effect upon a spontaneous tumor in the human patient.

SUMMARY

Because the doctrine of resistance has proved so barren and so inconsistent, it is proposed that propagable tumors be investigated from another aspect, and the relation between a tumor and its blood-vessels is suggested as perhaps worthy of consideration.

The question is raised whether the receding tumor may not differ from the growing one only in the extent to which its blood-vessels have been obliterated by thrombosis, and whether every growing tumor may not therefore be potentially a receding one.
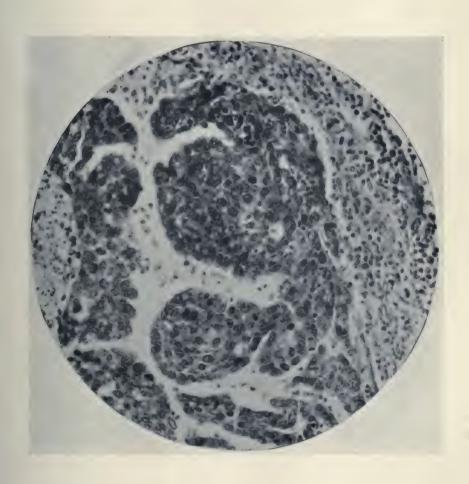
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PLATE 2

From the margin of a receding mouse carcinoma, no. 206, to show the presence of mitotic figures. Such fields as this are not difficult to find. × 500.

PLATE 5

A, a 21-day growing mouse carcinoma no. 48; B, a 52-day receding tumor of the same strain, which had been regressing for seven days. There are fewer small veins in the tissues about the regressing neoplasm, but this is not a constant characteristic of disappearing tumors.

UNUSUAL CARDIAC AND CEREBRAL METASTASES IN MELANOSARCOMA

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Clinical interest in cases of generalized melanosarcoma often becomes focused upon the extensive metastases in one or more organs. The primary neoplasm, if cutaneous, may have been forgotten by the patient or may show a relatively insignificant appearing recurrence at the site of the original tumor, which too often has been as much incised as excised, while the subcutaneous nodules and especially the luxuriant growth of the secondaries on hepatic soil are the outstanding features. Even at autopsy, the gross pathological picture may be such as to lead to serious misinterpretation of the primary site. There can be but little doubt that many of the reported instances of melanoblastomas arising in situations other than the eye and the skin are thus to be explained.

The case here presented is illustrative of the way in which the emphasis falls upon the secondary dissemination in cutaneous melanotic sarcoma. In addition, it presents an unusual coincidence of interesting features. The history of the case and the clinical findings failed entirely to indicate the extensive visceral metastases, and nothing in these suggested the remarkable meningeal involvement. A neoplastic nodule on the floor of the fourth ventricle, which had ruptured through the ependyma, did not influence the clinical findings sufficiently to attract attention. As for the heart, a moderate aortic regurgitation led a member (Dr. George R. Herrmann) of the Internal Medicine Staff to suggest the possibility of secondaries upon the aortic valve cusps, but this possibility was not taken seriously, and that the entire myocardium was studded with neoplastic nodules was unsuspected.

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In retrospect, one might well ascribe the nausea and vomiting which were present, to a central nervous system origin; the muscle pains to the hundreds of metastases in the skeletal muscles and muscle fascia, many of which must have encroached upon nerves, or to the diffuse infiltration of the spinal meninges; the periods of irrationality to the meningeal involvement; and the lung signs toward the end, which were clinically considered to be due to a terminal broncho-pneumonia, to the failing pulmonary circulation from destruction of the myocardium or to the numerous pulmonary metastases. On the other hand, there is not one of these findings which might not well receive some other clinical explanation when found in a patient dying, as this one was, in a condition of marked tumor cachexia.

CASE REPORT

The following clinical notes are abstracted from the records of the Department of Internal Medicine, service of Doctor Newburgh.

E. B., male factory laborer, age thirty-three, entered the University Hospital January 10, 1922, as an emergency patient. He was brought in on a stretcher and was too ill to give his history. His chief complaints were pain in the abdomen and back, vomiting, and weakness. From his wife it was learned that he had been well up to nine weeks before. At that time he was taken with severe pains in the muscles of the arms, back, and legs, and the hands and arms were swollen. He went to bed for ten days and the swelling disappeared. Several days later he developed a severe pain in the mid-epigastrium which was thought to be due to a gastritis. At first food or soda relieved the pain but later they seemed to have no effect. He was nauseated but had not vomited until three weeks before entrance. The severe vomiting spells which began at that time had no relation to meals and from time to time the vomitus was mixed with altered or even bright red blood. He had been unable to retain any food for about a week previous to entering the hospital. Small tumors began to appear over the body about three weeks before entrance and they seemed to be growing somewhat larger.

Family and personal history were without incident except that the patient had had a large pigmented mole removed from his back about two years before. The exact date was not known. Some time before

this operation he had fallen and traumatized a portion of the mole and this accident was followed by infection in the mass of the tumor. According to the wife a pathological diagnosis of "pigmented cancer" was made after the operation.

Nine weeks before entrance the patient weighed 164 pounds. He lost 34 pounds in the first four weeks of his illness and loss of weight had continued. He slept poorly and had been receiving morphine for the past two weeks. He had been slightly irrational for the past week.

Abstract of physical examination by Doctor Hills

The patient is poorly nourished. He is lying in bed, is very drowsy and does not respond to questions. He appears rather unclear mentally. The hair is dark brown in color and coarse in texture. The skin over the face appears slightly flushed; over the remainder of the body it is warm, moist, and elastic. Immediately beneath the skin over the thorax, abdomen, thighs, and to a lesser extent over the back, there are numerous small tumor masses, ranging in size from that of a small pea to that of a pinhead. The tumor masses are freely movable beneath the skin, are hard, and have a slight bluish tinge. In the lower right back there is a large scar, slightly bluish in color, which is the site of the operation done about two years before. The eyes are negative except that the pupils react sluggishly to light. There is no apparent disturbance of hearing. The thorax is long and narrow. On palpation of the cardiac region there is a marked shock felt over the area of the apex impulse. On auscultation, immediately following the first sound there is a rough systolic murmur slightly heard at the apex and especially well heard at the left border of the sternum. The first sound is very loud. Over the aortic area there is a faint diastolic murmur. The lungs show some dullness over both apices, extending down into the interscapular region. Over the entire abdomen there is a tenderness with some muscle spasm upon palpation.

The urine was practically negative except for a positive melanogen test. The blood showed 87 per cent hemoglobin, 4,300,000 red cells, and whites increasing from 17,000 to 26,200 during the stay of the patient in the hospital. The blood pressure was 160/60. The blood Wassermann was negative.

The provisional diagnosis was: Melanotic sarcoma, diffuse; aortic regurgitation; emaciation.

Following his admission to the hospital, the patient gradually grew worse, vomiting being so frequent that he retained practically no food.

He was irrational at times and very restless. On the morning of the 17th there were many râles on the right side and he was believed to be developing a broncho-pneumonia. He then grew rapidly worse and died the evening of the 18th.

Autopsy findings

(*Dr. C. V. Weller, prosector*)

Autopsy was done ten hours post-mortem. The more significant data are given in the abstract which follows.

The body is that of an adult male of slender build, 176 cm. long, showing marked emaciation. In the lower part of the right back there is a thin, freely movable, non-pigmented scar. At the upper end of this there is an elevated, deeply pigmented nodule, about 1 cm. in diameter, which has a bluish black color through the overlying epidermis. In the right axilla there is a smooth oval mass nearly the size of a hen's egg. Scattered everywhere in and beneath the skin there are very numerous small nodules, varying in size from shot-like grains, barely palpable, up to 1 cm. in diameter. These, in part, move freely with the skin but in part are more deeply situated and fixed, the skin moving over them. Most of these nodules show no pigmentation. Some of the larger, especially those which are situated near the epidermis, show a deep purplish or bluish color.

The spinal cord, examined only in the upper cervical region, shows no change except thickening of the meninges. Scalp and cranial vault are negative. The dura appears thicker than normal. The leptomeninges show everywhere a diffuse thickening, without pigmentation, and are more adherent to the cortex than normally. The cerebrum shows a moderate general congestion and edema. In the subcortical zone on the left side, about three cm. from the midline superiorly, there is a small area of softening in the brain substance. This has a pigmented, brownish border, not unlike that seen about areas of cerebral infarction with softening, and can not be positively known to be neoplastic by naked eye examination. On the floor of the fourth ventricle there is an elevated nodule, about 5 mm. in diameter and gray in color. This is evidently neoplastic.

The pericardial tension is normal, the pericardial fluid clear. The heart is somewhat larger than the cadaver's right fist. Scattered everywhere in and beneath the epicardium there are pale grayish-white to yellowish-white neoplastic nodules, ranging in size from minute

points, barely visible, up to 1 cm. in diameter. Most of these show no unusual pigmentation, although a few of the largest are brownish in the central portion. The right ventricular wall measures 7 mm. in thickness; the left, 14 mm. Metastatic nodules similar to those beneath the epicardium are found everywhere throughout the myocardium and in and beneath the endocardium. On the papillary muscles of the left ventricle they are very numerous, appearing elevated and slightly roughened on the surface. Here there is practically no evidence of pigmentation. Valvular orifices and valve flaps are negative except the aortic, which admits the thumb with ease. Near the edges of the aortic cusps there are several small nodular masses, possibly neoplastic. These are not pigmented and are firmer in consistency than the undoubted neoplastic nodules in the myocardium.

The autopsy findings in other organs may be summarized for the purpose of this report with the statement that the intercostal muscles, the substernal fascia, both lungs, omentum, peritoneum, spleen, pancreas, liver, kidneys, peri-adrenal tissue, bladder mucosa, and testes showed nodular metastases to the naked eye. The lungs showed no gross evidence of pneumonia, but there was a well marked congestion and edema.

Microscopical findings
(See below for detailed description)

Brain. Diffuse sarcomatous growth throughout the meninges. Large nodular metastasis in the floor of the fourth ventricle, showing central necrosis and a few pigmented cells. General congestion and edema.

Choroid plexus. Numerous metastases throughout.

Spinal cord meninges. Diffuse sarcomatous growth. Congestion and edema.

Heart. Numerous metastases in the endocardium, throughout the myocardium and in the epicardium (detailed description below). To some of the endocardial metastases fresh thrombi are attached, and between the trabeculae of the left ventricular wall there are large masses of recent mixed clot containing several areas filled with apparently living neoplastic cells.

Aorta. Slight sclerosis. The para-aortic lymph nodes are filled with metastases.

Lungs. Multiple metastases. Pigmented cells are found in small numbers in the older and larger metastases. None elsewhere.

Tonsils. Moderate atrophy. Hyperkeratosis.

Thyroid. Excess of colloid. Colloid cysts. Numerous metastases.

Spleen. Chronic passive congestion. Lymphoid atrophy. Numerous metastases, some with central necrosis and hemorrhage.

Pancreas. Numerous metastases.

Stomach. Slight atrophic catarrhal gastritis.

Intestinal tract. Chronic atrophic catarrh. Peritoneal surface covered with metastases and metastases even in the muscularis.

Mesenteric fat. Small metastases everywhere.

Adrenals. Small metastases in both cortex and medulla. Numerous metastases in peri-adrenal tissue.

Kidneys. Multiple metastases. Atrophy. Cloudy swelling. Acute congestion. Numerous casts. Sclerosis of arteries.

Testes. Diminished spermatogenesis. Some increase in stroma. Several metastases.

Prostate. Cystic glandular hyperplasia. Small metastases.

Bladder. Metastases in the mucosa and in the muscularis.

Recurrence in scar on back. A sarcoma of the type described below, showing relatively few pigmented cells in the greater part of its mass.

Type of tumor. In its recurrence and in the metastases, the neoplasm is an alveolar polymorphous cell sarcoma. Many areas have a highly vascular stroma and there are many large atypical giant cells. In portions of the recurrence there are numerous pigment-bearing cells. These are found in relatively large numbers, also, in the large axillary metastasis and in smaller metastases elsewhere, but a majority of the metastases show very little melanin. A chromatophoroma, but chiefly non-pigmented.

Pathological diagnosis

Recurrent melanosarcoma of the skin (primary in pigmented mole of lower right lumbar region, operated upon about two years previously). Multiple metastases in meninges, brain, lungs, bronchial nodes, heart, spleen, liver, kidneys, adrenals, bladder, prostate, testes, pancreas, peritoneum, intestinal wall, and all lymph nodes. Generalized sarcomatosis, chiefly non-pigmented. Atrophy, passive congestion, and parenchymatous degeneration of all organs. Aortic insufficiency due to sarcomatous infiltration of the myocardium. Tumor cachexia. General marasmus.

General character of the neoplasm

In view of the fact that the original pigmented mole was accidentally traumatized and then operated upon about two years before the patient entered the Hospital, it is of interest to note that the present illness was considered as of but nine weeks' duration. During the fore part of this long latent period the primary neoplasm may have been growing slowly without sufficiently heterotypic characteristics to give metastases. In view of the autopsy findings, however, another explanation can well be entertained. By far the largest metastasis found was that in the right axilla. It also showed the most marked degree of pigmentation. It is not at all unlikely that this may have been established before the original operation and that from it some, if not all, of the later general hematogenous dissemination may have been derived.

The extraordinary rapidity of growth, once numerous metastases were established, as shown by the clinical course, is indicated also by the relative lack of pigment. Many of the fairly good sized nodules might well be described as amelanotic, so that only the larger and older metastases fully reveal the pigment producing inheritance of the neoplasm. The foudroyant course of the overwhelming terminal sarcomatosis points to a sudden failure or exhaustion of some inhibiting power which had hitherto held blastomatous proliferation somewhat in check, or to a rapid exaltation of the proliferative energy of the blastoma cells.

In general, the type is that of an alveolar sarcoma exhibiting much variety in size and form of cells, but many of the smaller and presumably younger metastases and the emboli do not show the polymorphic type and are round celled. New formed blood vessels are abundant in some of the metastases, the appearance in such areas approaching that of an angio-sarcoma.

Brain and meningeal metastases

The neoplastic nodule on the floor of the fourth ventricle is of striking appearance, both in the gross and in microscopical sections. It was originally somewhat below the ventricular ependyma but with progressive growth ruptured through the overlying tissue, which is folded back upon itself on either
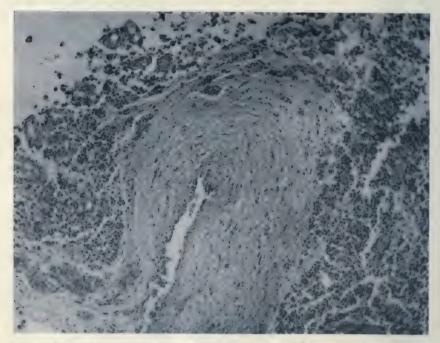
FIG. 1. PHOTOMICROGRAPH OF THE METASTASIS OF MELANOSARCOMA IN THE FLOOR OF THE FOURTH VENTRICLE

The main mass of the tumor nodule lies to the right. The more superficial neuroglia and ependyma have ruptured and are turned back toward the left. Note the nearly complete absence of pigment.

side, the ependymal layer being otherwise intact (see fig. 1). After thus breaking through, the neoplasm presents a ragged surface from which free blastoma cells might well have been given off into the ventricular fluid.

The meningeal dissemination constitutes the most remarkable feature of the case. The diffuse opacity, thickening and greater

degree of adhesion of the leptomeninges, as noted at autopsy, proved to be due to a diffuse sarcomatous infiltration, spreading everywhere through the inner meninges of both brain and cervical cord, and from the pia streaming down into the cortical and even subcortical tissue (see figs. 2, 3, and 4). The infiltration is not quite so abundant over the gyri, but the sulci are completely

Fig. 2. Low Power Photomicrograph of the Cerebral Leptomeninges to Show the Continuous Mass of Neoplasm Over the Surface and Streaming Down into the Cortex and Subcortical Tissue

Below, are older, heavily pigmented metastases having a very different manner of growth.

filled by the infolded blanket of new growth. The highly vascular meninges and outer cortex have furnished an exceedingly favorable soil for the growth of the tumor cells, and in the lower portion of the layer of sarcoma cells, actually in brain substance, a perivascular arrangement about the original cerebral vessels

becomes very evident. In this zone, also, are to be found
numerous groups of rather deeply pigmented cells which are
almost entirely perivascular in position. Curiously enough,
these melanin-bearing metastases are relatively small, and
although undoubtedly older than the more superficial masses,
as shown by the marked pigmentation, certainly exhibit a much

Fig. 3. Photomicrograph of the Lower Part of the Diffuse Meningeal
Sarcomatosis, Showing Its Cortical and Subcortical Extension

feebler growth than that luxuriantly infiltrating from the diffuse
meningeal layer.

It must be emphasized that the meningeal dissemination in
this case is exactly like that described for certain of the so-called
primary meningeal melanosarcomas. It bears a very close
resemblance, for instance, to the case reported by Lua (1) as
primary in the meninges, and shows a much more diffuse exten-
sion than that in the case of Schopper (2). Such a case as this,

in which a primary cutaneous melanosarcoma is unquestioned, throws very grave doubt upon the supposed primary meningeal origin of similar diffuse meningeal melanosarcomas.   The possibility of such an origin is accepted by many, including Ewing, the melanoblastoma supposedly having its origin in certain pigmented cells described as occurring normally in the lepto-

Fig. 4. Photomicrograph of the Deeper Cerebral Extensions from the Meningeal Metastases with a Few of the Small, but Much Older, Pigmented Cerebral Metastases

meninges.   Here, however, the same gross and microscopical picture is presented as in some of the so-called primary cases, so that it must be concluded that the occurrence of a primary meningeal melanosarcoma has not been fully demonstrated.

Fig. 5. Multiple Sub-epicardial and Myocardial Metastases of
Melano-sarcoma

Heart

The occurrence of myocardial metastases in melanotic sarco-
matosis is not unusual.   The heart in this case, however, showed
metastases in such extraordinary profusion (see fig. 5) that there
is no difficulty in bringing a number of them into a single low-
power field of the microscope.   Metastases are especially numer-
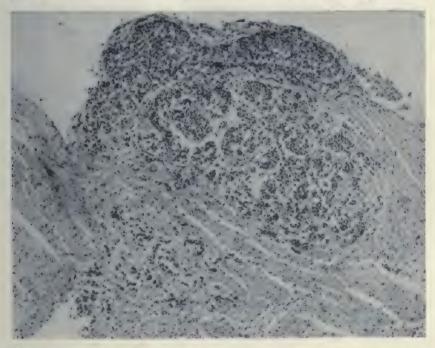
FIG. 6. PHOTOMICROGRAPH OF A SUB-ENDOCARDIAL METASTASIS OF MELANO-
SARCOMA WITH A MASS OF TUMOR CELLS GROWING ON THE
ENDOCARDIUM DIRECTLY ABOVE THE NODULE

ous in and beneath the endocardium, and it is frequently noted
that if a group of sarcoma cells lies just beneath the endocardium,
it is capped by other cells lying without the original endocardial
line and presenting themselves freely to the circulating blood
(see figs. 6 and 7).   To such plaques of cells mixed clot is often
found adherent, and from them the masses of neoplasm and
mixed clot lying nearly free in the ventricles were doubtless
derived.

The small nodules on the aortic cusps could not be distinguished positively at autopsy from the endocardial metastases. On microscopical examination, they are found to be composed of hyaline connective tissue without neoplastic cells, and are therefore not metastases. In the myocardium about the aortic ring, however, relatively large metastases are found. To the result-
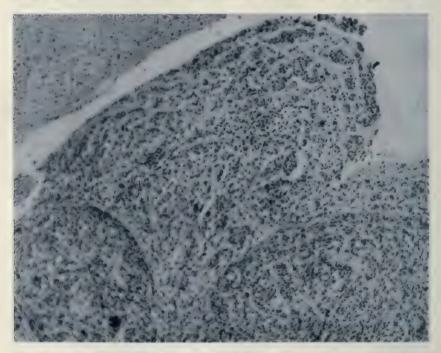
FIG. 7. PHOTOMICROGRAPH SHOWING PORTIONS OF TWO LARGER SUB-ENDOCARDIAL
METASTASES WITH A LARGE MASS OF NEOPLASM GROWING FROM THE
CORRESPONDING ENDOCARDIAL SURFACE INTO THE INTER-
TRABECULAR SPACE OF THE VENTRICLE

ing relaxation of the ring, the aortic insufficiency must have been due. The sclerotic nodules doubtless represent the healing of some older infectious process, and are inadequate in size to explain the aortic lesion.

SUMMARY

In a case of diffuse melanotic sarcomatosis dying two years after mechanical trauma to, and operation upon, a pigmented mole, there are found in the brain a solitary metastasis in the floor of the fourth ventricle, innumerable older cortical and sub-cortical metastases and a diffuse meningeal sarcomatosis, none of which had influenced the clinical picture in such a manner as to call attention to its existence. The meningeal involvement is exactly like that described for certain cases of alleged primary meningeal melanosarcoma, and throws further doubt upon the possibility of such origin. Very numerous myocardial and endocardial metastases were present, producing a relative aortic insufficiency clinically evident.

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THE INFLUENCE OF INORGANIC SALTS UPON TUMOR GROWTH IN ALBINO RATS

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There is some experimental evidence showing that some of the heavy metals and certain colloidal metals (1–17), especially those in which the ions of metals are powerfully germicidal, such as mercury, silver, copper, and arsenic, have a selective action upon cancer cells.

Since it is possible to affect definitely the virulence of tumor cells by chemical treatment outside the body, and since certain chemical agents have been believed by various investigators to be of value in the treatment of human cancer, we have felt that extended studies along the line of possible chemotherapy in cancer are imperative. For this purpose prolonged oral feeding of inorganic salts has many advantages over intravenous injection.

The systemic chemotherapeutic treatment of human cancer has been generally abandoned in recent years, probably because the amount of chemical agent required to produce a definite inhibitory effect upon the malignant cells is so great that general toxic effects are produced.

As far as we are aware, no investigations with experimental animals on the action of orally administered inorganic salts upon the natural immunity and upon the proliferative capacity of transplanted neoplasms have been reported. There is, however, a limited number of reports upon the effect of internally administered inorganic salts in cases of human cancer. Thus Carmichael (18) fed cancer patients with iron carbonate; Regnault (19) with magnesium chloride; Dubard (20) with magnesium carbonate; and Robins (21) with potassium nitrate.

EXPERIMENTAL

Throughout the present experiments we used the Flexner-Jobling rat carcinoma. The method of inoculation and of recording the progress of the transplanted tumors, was identical with the technic fully described by us in a previous article (22).

It was necessary to select a simple and an economical yet adequate food to carry out the laborious experiments. Therefore we chose cracker meal (National Biscuit Co.) and whole milk powder (Klim, Merrell-Soule Co.) as the basal diet. Preliminary feeding experiments with normal rats showed a uniform mixture of 32.1 parts of cracker meal and 5.1 parts of milk powder by weight to be an ideal diet for the growth, maintenance, reproduction, and perfect milk production of the albino rats. The completeness of the diet was further proved by the normal rate of growth of the transplanted Flexner-Jobling rat carcinoma in rats fed on this diet, controls being fed upon a common diet of bread, milk, and carrots.

The stock solutions of inorganic salts were prepared from chemically pure salts and distilled water. Compounds employed which do not dissolve in water, such as copper arsenite, zirconium oxide, and magnesium carbonate, were suspended in water and this suspension mixed with the food.

Each day a mixture of 50 parts of the basal diet and an equivalent amount of the salt solution was prepared. Thus the percentage of final salt content in the diet would be reduced to one-half of the initial strength of the solution or suspension used.

The amounts of food consumed, as well as the body weight of each animal, were recorded every third or fourth day.

Throughout the experiments the animals were maintained upon the special diets from seven to fourteen days previous to tumor inoculation so that the administered salts might have their full physiological effect both before and after inoculation with the tumor tissue. This procedure also served to permit the experimental animals to become accustomed to the new diet. As a control, the same number of animals of nearly equal body weight were fed with our basal diet and were inoculated with the same tumor tissue at the same time.

It has been clearly demonstrated (22–34) that malnutrition of hosts due to the lack of vitamines or other components of food causes an inhibition in the rate of tumor growth. Furthermore, we have shown that inadequacy of diet has no specific influence upon tumor immunity. It is for this reason that in addition to the observation of the progress of tumor growth we have made careful note of the general condition of the hosts fed with the various inorganic salts.

We employed generally a salt solution having three concentrations. The lowest concentration of a salt solution caused no harmful influence upon the body metabolism; while the higher concentration induced certain metabolic disturbance; and still higher concentration produced marked toxic action.

The composite results are presented in table 1. In the second column the concentration of the salt solution is given and in the third column the average amount of salt consumed daily during the experimental period, lasting from six to eight weeks for each animal. It must be remembered that the rats of different ages (we used animals, both males and females, from fifty to one hundred and fifty days old at the time of tumor inoculation) show a variation in the daily food consumption, and also a variation in the toxic effect of the salts. Therefore, the amount of salt taken in by the individual animal given in the third column represents the average, and was calculated from the amount of food eaten by at least ten rats for each experiment lasting more than three weeks. In the last column we give a brief statement of the animals' general condition. Since we did not make an extensive histological examination of the various organs of the animals, the harmful or non-harmful action of the metallic salts tested was indicated only by the general nutritive condition found by gross examination.

In connection with table 1 it may be pointed out that in certain instances an increase in the concentration of a given salt in the food may result in a generally poor condition of the animal although actually less of the salts is taken into the organism than where a weaker salt concentration was employed. This is apparently due to the higher concentration of salt affecting the total quantity of food eaten.

TABLE 1

Effect of orally administered inorganic salts upon the nutrition of albino rats

NAME OF SALT	CONCEN- TRATION OF SALT SOLUTION	AMOUNT OF SALT EATEN PER DAY	NUTRITION OF ANIMALS
	per cent	mgm.	
NaF...............	0.1	5.7	Animals maintained body weights, general appearance good
NaCl..............	2.0	100	Growth of animals was normal
	10.0	285	Animals maintained body weights; the normal health was somewhat poor toward the end of the experiment
NaI..............	1.0	46	The growth of animals was one third of normal rate; their general appearance was good
	1.5	63	Animals did not grow but maintained body weights; general appearance was good
NaH$_2$PO$_4$..........	2.0	110	The growth of animals was normal
	5.0	255	The growth of animals was one half of normal rate of growth; general ap-appearance was good
Na$_2$HPO$_4$..........	1.7	72	The growth of animals was normal
	3.0	220	The growth of animals was normal
NaHCO$_3$...........	2.0	103	The growth of animals was slightly sub-normal
	5.0	258	The growth of animals was one half of normal rate
K$_2$CO$_3$.............	2.0	153	The growth of animals was normal
	3.0	180	The growth of animals was normal
	4.0	396	The growth of animals was normal
	5.0	410	Animals did not grow, but maintained body weights; general appearance good
	10.0	470	Animals did not grow, but maintained body weights; some died on this diet
KNO$_3$..............	1.0	75	The growth of animals was two thirds of normal rate; general appearance good

TABLE 1—*Continued*

NAME OF SALT	CONCENTRATION OF SALT SOLUTION	AMOUNT OF SALT EATEN PER DAY	NUTRITION OF ANIMALS
	per cent	*mgm.*	
KNO₃	2.0	142	Most of animals did not grow, but maintained body weights; some gained a little; general appearance good
CuSO₄	0.005	0.37	The growth of animals was normal
	0.05	3.4	The growth of animals was one third of normal rate; general appearance good
	0.1	5.8	The growth of animals was one third of normal rate; general appearance good
	0.3	14.0	Animals did not grow but maintained body weights; general appearance good
	0.5	22.5	Animals did not grow, but maintained body weights; general appearance good
CuHAsO₃	0.04	2.6	Animals kept losing weight; general appearance poor
As₂O₃	0.04	1.3	Animals did not grow, but maintained body weights; some lost slightly; general appearance fair
	0.05	1.2	Animals kept losing weight; some died shortly after arsenic feeding
	0.075		A lethal dose
MgCl₂	1.0	67	The growth of animals was normal
MgCO₃	0.5	37	The growth of animals was normal
ZnSo₄	1.0	50	Animals lost weight slightly; general appearance good
HgCl₂	0.1	4.9	Animals did not grow, but maintained body weights; general appearance good
	0.2	7.3	Animals lost body weight slightly; general appearance good

TABLE 1—*Continued*

NAME OF SALT	CONCENTRATION OF SALT SOLUTION	AMOUNT OF SALT EATEN PER DAY	NUTRITION OF ANIMALS
	per cent	mgm.	
CaCl₂............	1.0	66	Animals did not grow, but maintained body weight; general appearance good
	2.0	117	Animals did not grow but maintained body weight; general appearance good
	5.0	98	Animals lost weight steadily; emaciated, died within forty-four days on this diet
Ca(C₂H₃O₂)₂.......	2.0	109	Animals did not grow, but maintained body weight; some lost weight; others died
SrCl₂..............	0.2	17.3	The growth of animals was normal
BaCl₂.............	0.1	6.9	The rate of body growth was one third of normal; general appearance good
MnSO₄...........	1.0	70	The growth of animals was one half of normal; general appearance good
FeCl₃.............	1.0	65	The growth of animals was slightly below normal; general appearance good
CoCl₂.............	0.05	2.4	Animals did not grow, but maintained body weight; general appearance poor
Na₂MoO₄..........	0.2	16.3	Animals gained weight slightly; general appearance good
Na₂WO₄...........	0.25	9.3	Animals kept losing body weight, emaciated; some died in twenty-five days
(UO₂)(C₂H₃O₂)₂.....	0.1	5.9	The growth of animals was normal
	0.5	30.3	The rate of body growth was one half normal; general appearance good
	1.0	45.5	Animals kept losing body weight heavily; general appearance very poor; died in fifty-four days

TABLE 1—*Concluded*

NAME OF SALT	CONCEN-TRATION OF SALT SOLUTION	AMOUNT OF SALT EATEN PER DAY	NUTRITION OF ANIMALS
	per cent	*mgm.*	
$Na_2S_2O_3$	2.0	128	The growth of animals was slightly below normal; general appearance good
H_2SeO_4	0.0025	0.24	The rate of body growth was one half normal; general appearance good
	0.005	0.35	Large animals did not grow, but maintained good health, while small animals did not live on this diet
H_2SeO_4	0.0025	0.12	The rate of body growth was two thirds of normal; general appearance good
$CuSO_4$	0.25	11.5	
$Te_2O_3(OH)NO_3$	0.001	0.06	The rate of body growth was one third of normal; general appearance poor; showed sign of losing body hair
	0.0025	0.13	Animals did not grow, but maintained body weight; general health poor; hair fallen out; mouth swollen; edema of eyelids
	0.005	0.25	Animals became very sick; died within ten days
GeO_2	0.1	5.7	The growth of animals was slightly below normal; general appearance good
$Pb(C_2H_3O_2)_2$	0.3	13.2	Animals did not grow, but maintained body weight; general health poor; some died on this diet
ZrO_2	0.2	9.8	The growth of animals was normal
OsO_4	0.05	4.3	The growth of animals was normal
	0.1	8.0	The rate of body growth was two thirds of normal; general appearance good

Another point of special interest in connection with table 1 is the very high toxicity which we have found for salts of selenium and tellurium. These salts are far more toxic for rats than any other salt we have studied. The lethal dose for selenic acid is about 0.35 mgm. per day (equivalent to 0.19 mgm. of selenium per day), while for tellurium the lethal dose is approximately 0.17 mgm. of the element per day. Thus tellurium stands as probably the most toxic substance known for rats. The general pharmacology of selenium and tellurium do not appear to have been studied in detail. Our finding of the practically complete loss of hair following the ingestion of minute quantities of tellurium is of interest.

In the earlier part of the present paper we cited a number of workers who reported that certain metallic salts had a specific effect upon tumor cells, while still others, from limited experimental results, claimed that internally administered salts increased the degree of natural immunity to cancer in man. Unfortunately the findings in our present experiments are contrary to such observations, and therefore do not support the theories advanced by certain investigators. Furthermore, with one exception, none of the 32 inorganic salts investigated possessed immunizing action, although certain salts when administered to animals in large quantity had a marked retarding influence upon the rate of growth of the transplanted rat carcinoma. Copper sulphate definitely reduced tumor susceptibility. On the other hand, the carbonate and chloride of magnesium had a small but distinct accelerating influence upon the development of the transplanted rat carcinoma.

In view of these facts we believe it is not necessary to reproduce the progress of the tumor growth graphically in every instance. We shall therefore show a number of typical examples graphically, where our results disagree with the results of others, or where our findings seem to be of definite interest.

Our complete experimental results are summarized in table 2.

TABLE 2

Results of transplanting Flexner-Jobling rat carcinoma in rats fed upon various inorganic salts

EXPERIMENT NUMBER	TUMOR TISSUE USED	NUMBER OF ANIMALS USED	NAME OF SALT	CONCENTRATION OF SALT SOLUTION	PERCENTAGE OF TUMOR TAKES	REMARKS
				per cent		
1	FRC 84A	9	Controls	0	78	Six tumors grew rapidly; one grew slowly; two retrogressed
		9	NaCl	2.0	67	Six tumors grew normally; three retrogressed
		9	KNO$_3$	1.0	78	Seven tumors grew normally; two retrogressed
2	FRC 87A	10	Controls	0	80	Seven tumors grew rapidly; one slowly; one retrogressed; one did not grow
		10	KNO$_3$	2.0	80	Eight tumors grew rapidly; two tumors retrogressed
		10	CuSO$_4$	0.3	60	Six tumors grew rapidly; four tumors retrogressed
		5	HgCl$_2$	0.1	100	One animal died 18 days after inoculation; tumors grew normally
3	FRC 100D	5	Controls	0	100	One tumor grew slowly; rest grew rapidly
		10	NaCl	10.0	100	One tumor grew slowly; rest grew rapidly
		10	NaCl	2.0	90	One tumor retrogressed; rest grew rapidly
		5	NaI	1.0	100	Rapid growths
		5	ZrO$_2$	0.2	100	One tumor grew slowly: rest grew rapidly
4	FRC 88B	5	Controls	0	100	Rapid growths
		5	(UO$_2$)(C$_2$H$_3$O$_2$)$_2$	0.1	100	Rapid growths
		5	Pb(C$_2$H$_3$O$_2$)$_2$	0.3	100	Tumors grew slowly

TABLE 2—*Continued*

EXPERIMENT NUMBER	TUMOR TISSUE USED	NUMBER OF ANIMALS USED	NAME OF SALT	CONCENTRATION OF SALT SOLUTION	PERCENTAGE OF TUMOR TAKES	REMARKS
				per cent		
5	$\dfrac{FRC}{89B}$	5	Controls	0	100	Four tumors grew rapidly; one showed retarded growth
		10	$(UO_2)(C_2H_3O_2)_2$	0.5	90	Nine tumors grew rapidly; one tumor retrogressed
		5	K_2CO_3	2.0	100	Normal growths at once
		5	$Pb(C_2H_3O_2)_2$	0.3	100	Normal growths at once
6	$\dfrac{FRC}{90A}$	5	Controls	0	100	Rapid growths
		10	$(UO_2)(C_2H_3O_2)_2$	1.0	90	Tumor growths were nearly normal; one tumor retrogressed
		5	$ZnSO_4$	1.0	100	Normal growths
7	$\dfrac{FRC}{83A}$	6	Controls	0	67	Two tumors retrogressed; others grew rapidly
		6	$CuSO_4$	0.005	100	Rapid growths
		6	$CuSO_4$	0.05	83	One tumor retrogressed; rest grew rapidly
8	$\dfrac{FRC}{83B}$	6	Controls	0	100	Rapid growths
		6	$CuSO_4$	0.1	50	Two tumors grew normally; one slowly; three retrogressed
9	$\dfrac{FRC}{85B}$	6	Controls	0	83	Five tumors grew rapidly; one, no growth
		6	$CuSO_4$	0.1	83	Five tumors grew rapidly; one retrogressed
		6	$CuSO_4$	0.3	17	One tumor grew slowly and ulcerated in the 5th week; five tumors retrogressed
10	$\dfrac{FRC}{88A}$	10	Controls	0	100	One tumor grew slowly; rest grew rapidly
		10	$CuSO_4$	0.5	60	Six tumors grew normally; three of them became necrotic and ulcerated early; three retrogressed; one tumor did not grow

TABLE 2—*Continued*

EXPERIMENT NUMBER	TUMOR TISSUE USED	NUMBER OF ANIMALS USED	NAME OF SALT	CONCENTRATION OF SALT SOLUTION	PERCENTAGE OF TUMOR TAKES	REMARKS
				per cent		
10	$\frac{FRC}{88A}$	10	$HgCl_2$	0.2	78	Seven tumors grew rapidly; one retrogressed; one did not grow
11	$\frac{FRC}{101E}$	5	Controls	0	80	Three tumors grew rapidly; one slowly; one retrogressed
		7	$CuSO_4$	0.3	71	Five tumors grew rapidly; one retrogressed; one tumor did not grow
		5	$MgCl_2$	1.0	80	One tumor retrogressed; other tumors grew little faster than control tumors
12	$\frac{FRC}{95B}$	5	Controls	0	100	Rapid growths
		5	As_2O_3	0.05	100	Tumors grew very slowly
13	$\frac{FRC}{96C}$	10	Controls	0	90	One tumor did not grow; others grew rapidly
		10	As_2O_3	0.04	100	All tumors grew slowly
14	$\frac{FRC}{97B}$	5	Controls	0	100	Rapid growths
		5	$CuHAsO_3$	0.04	100	Two tumors grew slowly; others grew normally
		5	$FeCl_3$	1.0	100	Rapid growths
		5	NaF	0.1	80	One tumor retrogressed; others grew normally
15	$\frac{FRC}{91B}$	5	Controls	0	60	Two tumors grew rapidly; one slowly; one retrogressed
		5	H_2SeO_4	0.0025	20	One tumor grew rapidly; rest retrogressed
		5	K_2CO_3	4.0	60	Three tumors grew rapidly; two tumors retrogressed

TABLE 2—*Continued*

EXPERIMENT NUMBER	TUMOR TISSUE USED	NUMBER OF ANIMALS USED	NAME OF SALT	CONCENTRATION OF SALT SOLUTION	PERCENTAGE OF TUMOR TAKES	REMARKS
				per cent		
16	FRC 91C	5	Controls	0	80	Four tumors grew rapidly; one tumor retrogressed
		10	H_2SeO_4	0.005	90	Seven tumors grew normally; two slowly; one retrogressed
		10	K_2CO_3	5.0	80	Seven tumors grew normally; one slowly; one retrogressed; one tumor did not grow
17	FRC 93A	5	Controls	0	100	Rapid growths
		5	H_2SeO_4	0.0025	100	Rapid growths
			$CuSO_4$	0.25		
		5	$Te_2O_3(OH)NO_3$	0.0025	100	Four tumors grew rapidly; one tumor grew slowly
18	FRC 93B	5	Controls	0	100	Rapid growths
		5	$Te_2O_3(OH)NO_3$	0.0025	100	Three tumors grew rapidly; two grew slowly
19	FRC 94A	10	Controls	0	100	Nine tumors grew rapidly; one grew slowly
		10	$Te_2O_3(OH)NO_3$	0.0025	100	One tumor grew slowly; rest grew rapidly
20	FRC 98A	5	Controls	0	100	Rapid growths
		5	OsO_4	0.05	100	Two tumors grew rapidly; others grew slowly
		5	$SrCl_2$	0.2	100	One tumor grew very slowly; rest grew narmally
		5	$CaCl_2$	1.0	100	One tumor grew normally, others slightly under normally
		5	$BaCl_2$	0.1	80	One tumor retrogressed; others grew rapidly

TABLE 2—*Continued*

EXPERIMENT NUMBER	TUMOR TISSUE USED	NUMBER OF ANIMALS USED	NAME OF SALT	CONCENTRATION OF SALT SOLUTION	PERCENTAGE OF TUMOR TAKES	REMARKS
				per cent		
21	FRC 99A	10	Controls	0	100	Rapid growths
		5	OsO_4	0.1	60	Three tumors grew rapidly; two tumors retrogressed
		10	$CaCl_2$	2.0	100	Rapid growths
22	FRC 101A	5	Controls	0	100	Rapid growths; showed growth of many secondary tumors
		10	NaI	1.5	80	Two tumors did not grow; others grew but slightly subnormally
23	FRC 95A	5	Controls	0	100	Four tumors grew rapidly; one grew slowly
		5	$CaCl_2$	5.0	100	All tumors grew very slowly
		5	K_2CO_3	10.0	60	One tumor grew rapidly; two tumors grew slowly; two retrogressed
24	FRC 101B	5	Controls	0	100	Rapid growths
		10	$MgCO_3$	0.5	90	One tumor retrogressed; others grew faster than control tumors
		10	K_2CO_3	10	90	One tumor retrogressed; others grew slowly
25	FRC 103A	10	Controls	0	100	Rapid growths
		10	$MgCO_3$	0.5	100	Tumors grew little faster than control tumor
		10	$Na_2S_2O_3$	2.0	100	Rapid growths
26	FRC 78A	10	Controls	0	80	Two tumors retrogressed; others grew rapidly
		8	$NaHCO_3$	2.0	88	One tumor retrogressed; two grew slowly; rest grew normally
		5	Na_2HPO_4	3.0	80	One tumor retrogressed; rest grew rapidly

TABLE 2—*Continued*

EXPERIMENT NUMBER	TUMOR TISSUE USED	NUMBER OF ANIMALS USED	NAME OF SALT	CONCENTRATION OF SALT SOLUTION	PERCENTAGE OF TUMOR TAKES	REMARKS
				per cent		
27	$\frac{FRC}{79A}$	5	Controls	0	80	One tumor did not grow; others grew normally
		5	NaH_2PO_4	2.0	60	Three tumors grew normally; two tumors did not grow
28	$\frac{FRC}{90C}$	5	Controls	0	80	Four tumors grew rapidly; one tumor retrogressed
		5	K_2CO_3	3.0	80	Four tumors grew rapidly; one tumor retrogressed
		5	$Ca(C_3H_5O_3)_2$	2.0	100	Three tumors grew rapidly; two grew slowly
29	$\frac{FRC}{103C}$	5	Controls	0	80	Three tumors grew rapidly; one grew slowly; one retrogressed
		10	NaH_2PO_4	5.0	30	Two tumors grew rapidly; one grew slowly; seven retrogressed
30	$\frac{FRC}{104C}$	5	Controls	0	80	Three tumors grew rapidly; one grew slowly; one retrogressed
		5	$NaHCO_3$	5.0	80	Three tumors grew rapidly; one grew slowly; one retrogressed
		5	Na_2HPO_4	3.0	40	Two tumors grew rapidly; three tumors retrogressed
		5	NaH_2PO_4	5.0	100	Rapid growths
31	$\frac{FRC}{105C}$	10	Controls	0	90	Nine tumors grew rapidly; one tumor retrogressed
		10	Na_2HPO_4	3.0	90	Nine tumors grew rapidly; one tumor retrogressed
32	$\frac{FRC}{96B}$	5	Controls	0	100	Rapid growths
		5	Na_2WO_4	0.25	100	Rapid growths
		5	$MnSO_4$	1.0	100	Rapid growths

TABLE 2—*Concluded*

EXPERIMENT NUMBER	TUMOR TISSUE USED	NUMBER OF ANIMALS USED	NAME OF SALT	CONCENTRATION OF SALT SOLUTION	PERCENTAGE OF TUMOR TAKES	REMARKS
				per cent		
33	FRC 97C	5	Controls	0	80	One tumor retrogressed; others grew rapidly
		5	$CoCl_2$	0.1	80	One tumor did not grow; rest grew normally
		5	Na_2MoO_4	0.2	80	Three tumors grew rapidly; one grew slowly; one tumor retrogressed
34	FRC 102D	5	Controls	0	100	Tumors grew somewhat slowly
		5	GeO_2	0.1	100	Tumors grew little faster than the control tumors

DISCUSSION OF RESULTS

Sodium chloride and potassium nitrate feeding

Bulkley in his recent book (35) emphasizes the importance of dietetic treatment of cancer. He believes that excess intake of sodium chloride is harmful, if it is not the possible cause of cancer, since it disturbs the salt equilibrium in blood. A similar supposition has been postulated before (36, 37). Robins, in his second paper (38), has pointed out that potassium nitrate would be the chemical to remove the excess sodium chloride in tissue. This empirical theory was tested in human cancer by the internal administration of potassium nitrate. He reported the treatment as successful.

Our present study was limited only to search for a substance which might influence the growth of cancer and not to study tumor etiology. Therefore we sought only to study the accelerating or retarding action of sodium chloride and potassium nitrate. To test these propositions rats were fed on our basal diet to which a definite amount of 2 or 10 per cent solution of sodium

chloride was added: or 1 or 2 per cent of potassium nitrate. Control animals were fed on our basal diet alone. At the end of the fourteenth day on these diets these rats were inoculated with tumor tissue and were fed on the same rations for six weeks longer. The complete results of these experiments are shown in

FIG. 1. EXPERIMENT 3

The rate of growth of the Flexner-Jobling rat carcinoma in rats fed 285 mgm. of sodium chloride daily is the same as the rate of the tumor growth in rats which did not take sodium chloride.

table 2, experiments 1, 2, and 3, and a typical example of each is graphically given in text figures 1 and 2.

In the table 1 is shown that the daily administration of 75 or 142 mgm. of potassium nitrate disturbed animal metabolism to a certain degree. With the former amount the daily body growth

of the rat was two-thirds of the normal; while with the latter amount the animal, as a rule, did not grow. From this fact we deduce that we have given potassium nitrate to rats in relatively greater amounts than did Robins to man, and therefore, the replacement of sodium chloride in tissue by means of a double decomposition with the purpose of substituting less harmful

FIG. 2. EXPERIMENT 2

The rate of growth of the Flexner-Jobling rat carcinoma in rats fed 142 mgm. of potassium nitrate daily is the same as the rate of the tumor growth in control rats.

potassium chloride in the cells should have been greater. Our experimental results do not verify Robins' findings, as potassium nitrate had neither immunizing influence in rats nor a retarding influence upon the growth of the transplanted cancer. It is also to be noted that this salt had no inhibitory influence on the appearance of secondary tumors.

Further proof of the inactivity of potassium nitrate upon the growth of tumor grafts is shown from the result of the following experiment. Twenty tumor-bearing rats were divided into two groups. Ten of them were placed on potassium nitrate diet (2 per cent solution of KNO_3) at the end of the fourteenth day after tumor inoculation, the time when grafted tumor begins to develop more rapidly. The other ten animals were allowed to remain on the basal diet. The tumors were allowed to grow four weeks more. The results of this experiment showed that the progress of the tumor grafts in the rats fed on both these rations were exactly identical and furthermore that not a single tumor had retrogressed. Thus we can infer that the potassium nitrate has no inhibitory action whatever.

Our results with sodium chloride feeding show that the salt exercises no influence upon the development of the grafted tumors.

Uranium acetate feeding

There arises the question, too, whether an artificially produced disease in the animal body can antagonize an already present malignant neoplasm. Benedict and Lewis (39) found that the transplanted Buffalo rat sarcoma will frequently retrogress from the effect of artificially induced phlorhizin glycosuria in rats. Their positive result was questioned by Wood and McLean (40) who used, however, only about one-sixtieth of the dose of phlorhizin employed by Benedict and Lewis.

Uranium salts have been commonly used to produce acute nephritis in animals. Opie and Alford (41) showed that uranium nitrate has a selective action on the kidney, causing necrosis of the renal tubules and leaving the parenchymatous cells of the liver and other organs relatively unaffected. According to MacNider (42) and Wilcox (43) the toxicity of uranium salts is constantly associated with its ability to induce a tissue acidosis.

We used uranium acetate to induce artificial nephritis in rats. Daily consumption of 5.9 mgm. of uranium acetate did not alter the normal rate of body growth; while intake of 30.3 mgm. of the salt daily reduced the rate of body growth to half of the normal.

The general appearance of these animals was good. On the other hand small animals consuming 45.5 mgm. of the salt daily lost body weight distinctly and died within fifty-four days. Histological examination showed that the kidneys of rats fed large quantity of uranium acetate were markedly degenerated.

FIG. 3. EXPERIMENT 6

Nephritis and acidosis produced by administration of uranium acetate has little or no influence on the rate of growth of the Flexner-Jobling rat carcinoma in rats.

Results obtained from tumor inoculation in these uranium acetate fed rats are presented in table 2, experiments 4, 5, and 6, and a characteristic example is shown graphically in text figure 3. It is clearly seen from these experiments that daily intake of 5.9 or 30.3 mgm. of uranium acetate had no influence upon the

tumor susceptibility and growth; and that in those animals given 45.5 mgm. of the salt the growth of tumor transplants was very slightly if at all retarded.   In other words the induced nephritis is ineffectual in retarding the proliferation of cancer cells.   Our result also indicates that tissue acidosis within certain limits has no influence upon the factors involved in tumor resistance.

Copper sulphate feeding

Some success has been reported in the treatment of human cancer through the use of colloidal copper.   Thus Loeb and his associates (10) reported that eight patients underwent repeated intravenous injections of colloidal copper solution and showed marked improvement in every way, and in some cases the tumor mass was distinctly diminished in size.   Additional observations (12, 14) on a larger number of treated patients confirmed their earlier conclusions, and they state that "intravenous injections of colloidal copper have a definite effect in a certain number of tumors, while in the case of others they are without any noticeable effect."   Moullin (15) noted that a nodule which was observed in the liver of a woman disappeared completely after injections of colloidal copper solution.   The success of this treatment was suggested as due to the fact that liver cells appear to have a specific affinity for copper salts.   In 1914 Fleisher and Loeb (13) made an extensive study on the influence of intravenously injected inorganic salts and inorganic colloids on the growth of mouse carcinoma.   Their results showed that copper nitrate, copper ammonium sulphate, mercuric chloride, gold sodium chloride, and lanthanum nitrate had no noticeable effect on tumor growth.   On the other hand, the colloids of copper, platinum, and gold, and certain combinations of copper and casein, showed a distinctly inhibiting reaction upon the growth of experimental tumor.   In this connection, however, it has been shown by Weil (44) that daily intravenous injections of a mixture of blood-serum and colloidal copper solution did not appear to exert a destructive action upon tumor tissue.   Chemical analysis of two tumors from treated patients failed to reveal the presence of copper.

Though copper sulphate has been little used in medicine it has been extensively used in the purification of water. It has a distinctly poisonous action toward lower organisms, and this partly by virtue of its property of precipitating albumin and combining with many of the constituents of the tissues.

In the present study we have determined the effect of administered copper sulphate on the rat carcinoma. For this we used several concentrations of copper sulphate solution as indicated in table 1. The results of tumor inoculations in copper sulphate fed animals are shown in table 2, experiments 2, 7, 8, 9, 10, and 11. It will be noticed that the daily administration of 0.37 or 3.4 mgm. of copper sulphate had no effect upon the progress of transplanted tumors. The former amount of copper sulphate did not disturb the body metabolism as indicated by the normal rate of body growth and the perfect health of the animals; while 3.4 mgm. of copper sulphate per day caused a reduction in the rate of the daily body growth to one-third of the normal. On the other hand the daily intake of 5.8 mgm. of copper sulphate or a larger amount often not only retarded the growth of transplanted rat carcinoma, but also diminished the susceptibility of rats to the tumor. It is also interesting to note that the tumor necrosis and ulceration in those animals fed copper sulphate occurred generally much earlier than the tumors of control animals (text fig. 4). Plate 1 shows the result of copper sulphate feeding on the growths of the body and tumor. The normal rate of body growth of rat fed 0.37 mgm. of copper sulphate is contrasted with much retarded growth of both tumor and host when fed 14 mgm. of copper sulphate daily.

Whether or not copper sulphate has any immunizing action is uncertain; but we are quite sure that the cause of reduced growth of Flexner-Jobling rat carcinoma is due to the selectivity of the copper salt and not secondarily to the malnutrition of the hosts. If the decreased tumor susceptibility and retarded tumor growth were due primarily to the general impaired health of the hosts, then the results from feeding other equally toxic salts would be identical. But our experiments have shown that none of the other salts we tried possessed any definite immunizing action,

though certain salts when used in large amounts had a retarding influence upon the tumor growth; but this action is in many instances not proportional to the toxicity of the salt given.

FIG. 4. EXPERIMENT 10

Internal administration of 22.5 mgm. of copper sulphate daily made animals more resistant to the inoculation of Flexner-Jobling rat carcinoma. This amount of salt had also a retarding influence upon tumor growth.

Arsenic trioxide feeding

Gaylord (45) has reported that arsenic pentoxide has rapid healing action upon thyroid cancer of the salmonides. He demonstrated the result by adding very small amount of arsenic pentoxide to the water in which the diseased fishes were living. Funk (46) observed that the intrapectoral injections of arsenious acid has a slight inhibiting effect on the growth of chicken sarcoma.

The trioxide of arsenic when dissolved in water gives arsenious acid; the arsenious ions thus formed are very toxic. A ration containing 50 grams of our basal diet and 50 cc. of 0.075 per cent solution of arsenic trioxide was found to be a lethal mixture. On the other hand, the animals maintained body weight and general health to a fair degree on a ration containing 0.02 per

Fig. 5. Experiment 13

The rate of growth of the Flexner-Jobling rat carcinoma in rats fed 1.3 mgm. of arsenic trioxide daily is markedly reduced.

cent of arsenic trioxide. Histological examination of the viscera of these arsenic fed rats at the end of the forty-ninth day showed that the lungs and heart were normal; the spleen was congested; and the liver showed fatty and granular degeneration, which was generalized, but not very intense. The kidneys showed chronic congestion, with moderate granular tubular degeneration.

The results of tumor inoculation in the arsenic fed rats were characterized by retardation of the tumor grafts. The substance, however, had no action upon the tumor susceptibility (table 2, experiments 12 and 13, text fig. 5).

Selenic acid and tellurium nitrate feeding

The therapeutic value of colloidal selenium and tellurium in treating cancer was much discussed soon after the report of v. Wassermann and his associates (2). Their positive observations were confirmed by Szécsi (7), Werner and Szécsi (8), and Watson-Williams (17). Pentimalli (47) has made a careful study of the effects of aromatic selenium compounds and found they have no curative action. But with eosin-selenium he obtained a cure in from one to two per cent of the treated animals. On the other hand, Uhlenhuth, Dold and Bindseil (48), Contamin, Detoeuf and Thomas (49), and Delbert (50) reported that eosin-selenium has no curative action on experimental and human cancer. Similarly Walker (51) had shown that the colloidal selenium, with or without eosin, possessed no destructive action upon the tumors in either rats or mice.

Selenic acid is a powerful oxidizing agent. The non-activity of this highly toxic chemical upon the rat cancer is shown in table 2, experiments 15, 16, and 17, and graphically demonstrated in text figure 6.

As mentioned earlier in this paper, tellurium nitrate, like selenic acid, is a very toxic substance. Thus a daily dose of 0.13 mgm. of the salt caused the body hair to fall out as early as on the fifth day. Swollen mouth, edema of eyelids, and emaciation in many rats were produced by the further administration of the salt. The loss of body hair, swollen mouth, and eye infections are transient, since return to normal could be brought about by the discontinuing of the tellurium nitrate feeding.

The results of tumor inoculation in tellurium nitrate fed rats are shown in table 2, experiments 17, 18, and 19, and graphically in text figure 7. It was found that the proliferation of cancer cells was neither influenced by the salt directly nor by the result

of marked external and internal body disturbance of the hosts from the extreme toxicity of the tellurium nitrate.

The experiments with tellurium feeding are of very definite interest in connection with certain aspects of the problem of the growth of cancer cells.   As mentioned earlier in this paper, it

FIG. 6. EXPERIMENT 16

Oral administration of 0.35 mgm. of selenic acid daily had no influence on the rate of growth of the Flexner-Jobling rat carcinoma in rats, even though the health of the hosts was greatly disturbed by this toxic substance.

has been definitely shown by a number of investigators that certain deficiencies in diet will result in retarded growth of a transplanted tumor and in a generally poor nutritive condition of the animal.   We report similar findings for copper sulphate, arsenic trioxide, calcium chloride, and potassium carbonate

feeding in the present paper. Copper sulphate also reduced the
susceptibility of the rats to the tumor transplant. In all such
animals, however, the general condition is very poor, and appar-
ently we are dealing with a non-specific effect upon the tumor—
an effect which is due simply to the general poor condition of the

FIG. 7. EXPERIMENT 19

The rate of growth of the Flexner-Jobling rat carcinoma in rats fed 0.13 mgm.
of tellurium nitrate daily is the same as the rate of tumor growth in control rats.

host. Our results with tellurium feeding lead us to question the
correctness of such a view. Tellurium causes a most marked
effect upon the general condition of the animal. Growth ceases,
the hair falls out, eye infections develop, etc. Yet these ema-
ciated, denuded, undersized animals will show just as good a
growth of cancer tissue as do those in a state of perfect health.

In plates 2 and 3 we have reproduced photographs of our tellurium fed rats. In these pictures are clearly shown a large tumor which was growing normally in a host which was in as poor a general condition as can be imagined. Such a result seems to indicate that there actually are some fundamentally different conditions governing the maintenance and growth of somatic and tumor cells.

Osmium tetraoxide feeding

Like selenic acid, "osmic acid" is a strong oxidizing substance as well as a powerful bactericide. Daily dose of 4.3 mgm. of osmium tetraoxide had no influence upon the nutrition of animals; on the other hand the dose of 8.0 mgm. slightly retarded the rate of normal body growth. The results of tumor transplantation showed that again this oxidizing substance failed to show any retarding or accelerating influence upon the tumor proliferation (table 2, experiments 20 and 21).

Sodium iodide feeding

One of the problems in the field of cancer chemotherapy is to find a substance which will penetrate a tumor mass when the animal bearing it is treated by intravenous or subcutaneous injections or by oral administration. Certain experiments reveal that iodide has a specific affinity for tumor cells. von den Velden (52) found iodine in metastatic carcinoma of the liver and pancreas after administration of one gram of sodium iodide for every twenty kilograms of body weight of the patient. Weil (53) has shown that the necrotic areas of tumors contain a larger amount of iodine than do the other tissues of the body of the rat after the intravenous injection of sodium iodide.

Iodides of sodium and potassium are used extensively in the treatment of tertiary syphilis. Cushny (54) states that "the specific effects of iodide in tertiary syphilis are exerted not on the parasite but upon the tissues in which it lives, and which have reacted to its presence by the formation of tumors; these lowly organized tumors dissolve under the action of iodides, while the parasite remains unaffected." Since there is direct evidence

that sodium iodide has a selective and autolytic action we believed
that a careful study of the effects of oral administration of sodium
iodide upon an experimental tumor would be of interest in the
present study.   The results are shown in table 2, experiments
3 and 22.   Although daily administration of 63 mgm. of sodium

FIG. 8. EXPERIMENT 22

Administration of 63 mgm. of sodium iodide daily to rats has little if any
influence on the rate of growth of the Flexner-Jobling rat carcinoma.

iodide stopped the body growths of animals it had very slight
retarding influence upon the normal rate of tumor growth (text
fig. 8).

Calcium chloride feeding

In a recent communication (55) it was clearly demonstrated
that the immersion of tumor tissue in a calcium chloride solution

(0.078 molar) at pH 7.0 for twenty-four hours destroyed its proliferating power. This suggested the desirability of determining whether such results could be duplicated in vivo.

We found that a mixture of 50 grams of our basal diet and 50 cc. of five per cent calcium chloride solution was an unsuitable ration for young rats. The animals lost body weight rapidly and died within 44 days upon this ration. The results of tumor inoculation in those rats can be briefly summarized as follows: First, the tumors developed after inoculation in 100 per cent of cases; and second, the growth of the tumors was markedly retarded (table 2, experiment 23). The harmful influence of calcium chloride upon the nutrition of the tumor and its host was checked when the concentration of calcium chloride was lowered. Although the body growth of animals was one-eighth the normal rate on the ration containing one per cent of calcium chloride, the growth of the tumors was normal (table 2, experiments 20 and 21). We can infer from these results that orally administered calcium chloride probably has a slight selective action upon tumor growth.

Magnesium chloride and magnesium carbonate feeding

It has been reported that cancerous growths will be retarded and often disappear under the influence of magnesium salts. Thus Regnault (19) stated that by the internal administration of magnesium chloride he was able to cure papilloma and superficial epithelioma. In the cases of inoperable cancer this treatment was not encouraging; but in some cases tumors showed marked retrogression. The beneficial action of the magnesium chloride according to this writer was explained by its action in stimulating phagocytosis. Dubard (20) gave to cancer patients magnesium carbonate from 8 to 12 grams daily after operations. He reports encouraging results. He postulates that the loss of magnesium in the body seems to favor the onset and development of malignant disease, and the administration of magnesium serves to restore this loss and exerts an inhibitory influence upon tumors. On the other hand Itami (56) has conclusively shown that intravenous injection of a magnesium chloride solution was

without effect on two types of mouse tumors. Further he has shown that the injections of magnesium chloride solution did not prevent the recurrence of spontaneous neoplasms.

FIG. 9. EXPERIMENT 24

Daily administration of 37 mgm. of magnesium carbonate has a slight but distinct accelerating influence upon the growth of the Flexner-Jobling rat carcinoma.

The present writers have studied the therapeutic value of chloride and carbonate of magnesium by using the method of oral administration. The results of our experiments differ from the above mentioned investigators in one respect. That is,

both carbonate and chloride of magnesium had a slight but distinct accelerating influence upon the growth of the Flexner-Jobling rat carcinoma (table 2, experiments 11, 24, and 25, and text fig. 9). This influence must be due to the magnesium ions, since neither carbonate nor chloride ions were found to possess such action. It will be recalled that magnesium is an essential constituent of food; and that the nutrition of the host has an influence upon the rate of the tumor growth. Since our basal diet contains enough magnesium for the normal metabolism of the animal the excess magnesium salts must have had a favorable influence in the cell proliferation of the tumor.

Primary and secondary sodium orthophosphate, sodium bicarbonate, and potassium carbonate feeding

During the course of the present investigation we have studied the effects of salt solutions at different hydrogen ion concentrations upon the proliferating capacity of the Flexner-Jobling rat carcinoma in vitro (55).

The results of this study can be briefly summarized below. Immersion of tumor tissues for twenty-four hours in a solution at pH 7.0 had no inhibitory effect upon the proliferating capacity of the tumor tissues. There was a slight inhibitory action at pH 8.0. Complete destruction of the viability of the malignant cells was obtained at pH 5.1, 5.5, and 8.8. These pH solutions were prepared with primary potassium orthophosphate and potassium hydroxide.

We have fed various phosphates to rats and studied the possible effect on tumor growth. The complete results are shown in table 2, experiments 5, 15, 16, 23, 24, 26, 27, 28, 29, 30, and 31. It is clearly shown that the prolonged administration of these salts to rats has no effect upon the tumor susceptibility or the rate of the growth of the transplanted tumors. Some examples are graphically represented in text figures 10 and 11. However, only when very high concentration of potassium carbonate was used, in which case the animal ultimately died from the results of

this feeding, was any retardation in tumor growth noticeable (text fig. 12).

Similar experiments were carried out with a large number of inorganic salts; namely NaF, $CuHAsO_3$, $ZnSO_4$, $HgCl_2$, $Ca(C_3H_5O_3)_2$, $SrCl_2$, $BaCl_2$, $MnSO_4$, $FeCl_3 \cdot CoCl_2 \cdot Na_2MoO_4 \cdot$

Fig. 10. Experiment 26

The normal rate of growth of the Flexner-Jobling rat carcinoma in rats is not altered by the daily feeding of 103 mgm. of sodium bicarbonate.

Na_2WO_4, $Na_2S_2O_3$, GeO_2, $Pb(C_2H_3O_2)_2$, and ZrO_2. These compounds have certain biological interest but detailed discussion is not necessary here since none of these salts as we employed them appears to possess any specific influence upon the tumor growth. The results are given in table 2.

Fig. 11. Experiment 16

The rate of growth of the Flexner–Jobling rat carcinoma in rats fed 410 mgm. of potassium carbonate daily is the same as in the control animals.

Fig. 12. Experiment 24

The rate of growth of the Flexner–Jobling rat carcinoma in rats fed 470 mgm. of potassium carbonate daily is somewhat below that of the controls.

SUMMARY

1. The possible therapeutic value of orally administered inorganic salts for the Flexner-Jobling rat carcinoma has been studied. The study included 32 different inorganic salts.

2. Copper sulphate, arsenic trioxide, potassium carbonate, and calcium chloride showed a retarding influence upon the growth of the tumor, but such action is not marked. Copper sulphate is the most effective agent in this respect, and appears to have some immunizing action against the tumor.

3. Tellurium nitrate and selenic acid have a very marked toxic action upon rats; but these compounds show no influence whatever upon the proliferating power of the tumor cells.

4. Magnesium carbonate and magnesium chloride show a slight but distinct accelerating influence upon the tumor growth.

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PLATE 1

The normal rate of growth of the Flexner-Jobling rat carcinoma as well as the normal rate of body growth of rats fed 0.37 mgm. of copper sulphate, are contrasted with the much retarded growth of both tumor and host when fed 14 mgm. of copper sulphate daily.
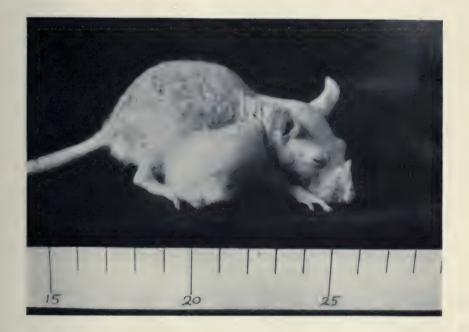
PLATE 2

Tellurium nitrate, though markedly inhibiting the growth of the host, is with-
out influence on the growth of the Flexner-Jobling rat carcinoma. The smaller
rat received 0.13 mgm. of tellurium nitrate daily for thirty-six days, while the
larger rat received no tellurium nitrate. The animals were the same age when
photographed.

The edema of eyelids, nearly complete removal of body hair, and ill appearance of the rat is solely the result of tellurium nitrate. The well developed tumor in such an animal demonstrates that tumor growth may be quite independent of the general condition of the host.

THE RELATIONSHIP OF CELLULAR DIFFERENTIA-TION, FIBROSIS, HYALINIZATION, AND LYMPHOCY-TIC INFILTRATION TO POSTOPERATIVE LONGEVITY OF PATIENTS WITH SQUAMOUS-CELL EPITHELIOMA OF THE SKIN AND LIP[1]

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The 98 cases of squamous-cell epithelioma of the skin and lip under discussion have been previously studied and graded by Broders. They represent all of the patients treated surgically at the Mayo Clinic for squamous-cell epithelioma of the skin and lip between November 1, 1904, and July 22, 1915, who died from recurrence of the lesion, or from metastasis.

The study of cellular differentiation, fibrosis, hyalinization, and lymphocytic infiltration was undertaken in an endeavor to determine the histologic factors causing the great variations in postoperative longevity (2, 3, 4, 5). Microscopic study of sections taken from many epitheliomas which were operable, and from others that were inoperable has revealed the presence of malignant cells taking on forms which closely resemble normal squamous-cell epithelium. They are well differentiated, as shown by the presence of pearly bodies. This phenomenon is termed cellular differentiation (fig. 1). In a great many of the cases the fibrous connective-tissue cells are seen working into and around the malignant tumor and apparently forming a barrier; this condition is known as fibrosis (fig. 2). Another phenomenon commonly seen is the presence of lymphocytes

scattered intimately around the tumor cells, in some instances in such abundance that it is difficult to find the malignant cells. The abnormal presence of lymphocytes around a tumor is believed to be lymphocytic infiltration (fig. 3). A condition less

FIG. 1. CELLULAR DIFFERENTIATION IN A CASE OF EPITHELIOMA OF THE SKIN. × 100
Two pearly bodies stand out prominently

FIG. 2. AREAS OF MALIGNANT CELLS COMPLETELY SURROUNDED BY DENSE FIBROUS CONNECTIVE TISSUE CELLS. × 100

commonly present is that of hyalinization (fig. 4) shown by a translucent or homogeneous condition of the connective tissue in and around the tumors.

Of the 32 patients with squamous-cell epithelioma of the skin treated surgically at the Clinic between November 1, 1904, and

FIG. 3. LYMPHOCYTIC INFILTRATION AS SHOWN BY THE LYMPHOCYTES IN AND AROUND MALIGNANT CELLS. × 100.
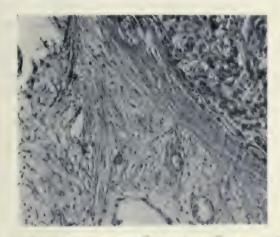
FIG. 4. HYALINIZATION WITH EPITHELIOMA CELLS. × 100

July 22, 1915, who are known to have died from recurrence of the lesion or from metastasis, complete data were obtained from 29. The tumors were sectioned and studied microscopically

without reference to clinical data; comparisons were drawn and deductions made as follows:
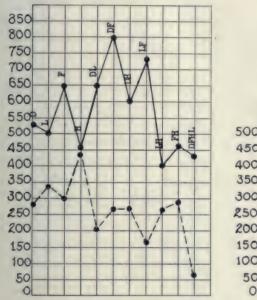
The average length of postoperative life was 444.6 days. The frequency of cellular differentiation was 65.5 per cent; of lymphocytic infiltration, 65.5 per cent; of fibrosis, 41.3 per cent; of hyalinization, 31 per cent; of cellular differentiation and lymphocytic differentiation combined, 37.9 per cent; of cellular differentiation and fibrosis combined, 27.5 per cent; of cellular differentiation and hyalinization combined, 17.2 per cent; of lymphocytic infiltration and fibrosis combined, 24.1 per cent; of lymphocytic infiltration and hyalinization combined, 20.6 per cent; and of fibrosis and hyalinization combined, 27.5 per cent.

The average length of postoperative life with cellular differentiation was 534.1 days; without cellular differentiation, 274.7 days (fig. 5); with lymphocytic infiltration, 496.2 days; without lymphocytic infiltration, 346.6 days; with fibrosis, 655.7 days; without fibrosis, 295.6 days; with hyalinization, 449.6 days; without hyalinization, 437.9 days; with cellular differentiation and lymphocytic infiltration, 644.5 days; without cellular differentiation and lymphocytic infiltration, 204 days; with cellular differentiation and fibrosis, 808.3 days; without cellular differentiation and fibrosis, 257.5 days; with cellular differentiation and hyalinization, 587 days; without cellular differentiation and hyalinization, 257.5 days; with lymphocytic infiltration and fibrosis, 739.8 days; without lymphocytic infiltration and fibrosis, 155.2 days; with lymphocytic infiltration and hyalinization, 404 days; without lymphocytic infiltration and hyalinization, 255.5 days; with fibrosis and hyalinization, 453.8 days; without fibrosis and hyalinization, 282.5 days; with cellular differentiation, lymphocytic infiltration, fibrosis and hyalinization, 444.6 days, and without cellular differentiation, lymphocytic infiltration, fibrosis and hyalinization, 54 days.

The average age of the 29 patients was 60.7 years. The oldest was 77 years, the youngest, 42 years. There were 25 males and 4 females. The average duration of the preoperative lesion was 6.1 years.

The lesions were in the head and neck in 22 cases; in the buttocks and sacral region in 3, in the extremities in 3, and in the abdomen in 1.

Of the 66 patients operated on for squamous-cell epithelioma of the lip between November 1, 1904, and July 22, 1915, all are

FIG. 5. AVERAGE LENGTH OF POSTOPER-
ATIVE LIFE WITH AND WITHOUT
FACTORS (EPITHELIOMA
OF THE SKIN)

FIG. 6. AVERAGE LENGTH OF POSTOPER-
ATIVE LIFE WITH AND WITHOUT
FACTORS (EPITHELIOMA
OF THE LIP)

Solid line indicates duration of posoperative life with factors present.
Dotted line indicates duration of postoperative life without factors present.

known to have died from recurrence of the disease, or from metastasis.   Data were obtained from 63 of these:

The average length of post-operative life was 359.8 days. The frequency of cellular differentiation was 71.4 per cent; of lymphocytic infiltration, 92.1 per cent; of fibrosis, 6.3 per

cent; of hyalinization, 6.3 per cent; of cellular differentiation and lymphocytic infiltration, 66.6 per cent; of cellular differentiation and fibrosis, 6.3 per cent; of cellular differentiation and hyalinization, 4.7 per cent; of lymphocytic infiltration and fibrosis, 4.7 per cent; of lymphocytic infiltration and hyalinization, 4.7 per cent; of fibrosis and hyalinization, 4.7 per cent.
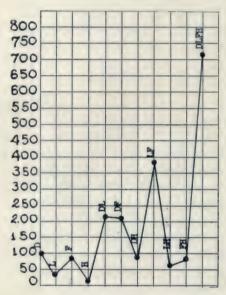
Fig. 7. Percentage Increase in Post-operative Life with Factors Checked against Postoperative Life without Factors (Epithelioma of the Skin)
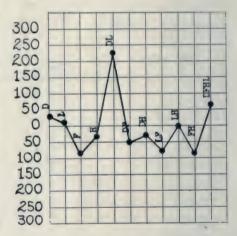
Fig. 8. Percentage Increase in Post-operative Life with Factors Checked against Postoperative Life without Factors (Epithelioma of the Lip)

The average length of postoperative life with cellular differentiation was 388 days; without cellular differentiation, 290.4 days (fig. 6); with lymphocytic infiltration, 365.9 days; without lymphocytic infiltration, 293 days; with fibrosis, 186.7 days; without fibrosis, 371.8 days; with hyalinization, 293.7 days; without hyalinization, 364.7 days; with cellular differentiation and lymphocytic infiltration, 386.8 days; without cellular differentiation and lymphocytic infiltration, 116 days; with cellular

differentiation and fibrosis, 186.8 days; without cellular differentiation and fibrosis, 290.4 days; with cellular differentiation and hyalinization, 207.6 days; without cellular differentiation and hyalinization, 275 days; with lymphocytic infiltration and fibrosis, 174.6 days; without lymphocytic infiltration and fibrosis, 310.5 days; with lymphocytic infiltration and hyalinization, 317.3 days; without lymphocytic infiltration and hyalinization, 310.5 days; with fibrosis and hyalinization, 207.6 days; without fibrosis and hyalinization, 367.1 days; with cellular differentiation, fibrosis, hyalinization and lymphocytic infiltration, 200 days; and without cellular differentiation, fibrosis, hyalinization, and lymphocytic infiltration, 117 days.

The average age of the 63 patients was 59.2 years. The oldest was 97 years, the youngest, 25 years. There were 61 males and 2 females. The average duration of the preoperative lesion was 3.57 years.

CONCLUSIONS

The average length of postoperative life of patients with epithelioma of the skin is increased when the factors differentiation, lymphocytic infiltration, fibrosis, and hyalinization are present. Postoperative life is increased when any one of these factors or a combination of them is present in the skin (fig. 7). However, this is not true in cases of epithelioma of the lip in the series studied. In these cases, postoperative life was increased when the factors cellular differentiation or lymphocytic infiltration were present (fig. 8); but with the factors fibrosis or hyalinization singly or in combination, postoperative life was decreased. This discrepancy may be owing to the fact that in the series of cases of epithelioma of the lip there were only 4 of fibrosis and 4 of hyalinization. Three of the patients with fibrosis also had hyalinization. One patient was 76 years of age, one was 68, and the other was 48, but had had the lesion for 11 years previous to operation. One patient with fibrosis alone was 70 years of age. The 1 with hyalinization alone was 58 years of age and had had the lesion 9 years previous to operation. Of the 4 patients with fibrosis, 3 had lived an average lifetime and

the fourth, although but 48, had had the lesion for 11 years and, therefore, his resistance was diminished. Two of the patients with hyalinization had lived 76 and 68 years respectively. The other 2 had had lesions 11 and 9 years respectively, and the defense offered by hyalinization must have been exhausted.

Despite the fact that with fibrosis and hyalinization there was a decrease in postoperative life in the cases of epithelioma of the lip, the presence of all the factors checked against the absence of all the factors showed an increase of postoperative life.

A larger series of cases should be studied before deciding that cellular differentiation, lymphocytic infiltration, fibrosis, and hyalinization, when in association with malignancy, prolong postoperative life as an entity, but from the data obtained from these 92 cases it would seem that each of these factors should be considered as a defense in cases of malignancy.

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THE REGRESSION OF SPONTANEOUS MAMMARY
CARCINOMA IN THE MOUSE

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There have been set aside at the Crocker Institute, during the past ten years, 2041 mice for the observation of what appeared to be spontaneous neoplasms. In a few instances the lesion proved later to be non-malignant, so that there have actually been available for study some 2000 tumors. Among these there have been 13 which regressed (fig. 1, and 905, fig. 2), and 3 which fluctuated or remained stationary in size (fig. 2). These 16 tumors occurred in 15 mice. That is to say, in 0.8 per cent among some 2000 mice, spontaneous carcinomata were unable to pursue their usual progressive course.

Murray (1) regards the absorption of part of a spontaneous tumor as a not uncommon phenomenon, but says that the complete disappearance of such a growth is a very rare event, as, indeed, it is. He has frequently seen, however, temporary arrest of growth, and in some cases actual diminution in size.

Apart from these statements, the only study of recession in spontaneous mouse tumors known to the writer is that of Haaland (2). In his series of 353 tumors, 5 or 1.4 per cent, regressed more or less completely.

When the two groups are averaged, it appears that approximately 1 per cent of spontaneous mouse carcinomata remain stationary or recede—an infinitely larger proportion than in man.

When a spontaneous mouse carcinoma begins to diminish in size, the investigator is torn between a desire to excise it for histological examination before it has disappeared entirely, and the consciousness that he ought to let it pursue its course un-

disturbed in order to be sure that it really would have been completely absorbed. In the present series, a number of the neoplasms were extirpated, while in the remainder of the cases the mice died before their growths had entirely regressed; there is but one instance (905, fig. 2) in which the tumor went on to complete absorption, and here, as in Haaland's series, it recurred later. Nevertheless, it is felt that, in the other cases, regression was allowed to proceed far enough to make it probable that the tumors would ultimately have disappeared. The most common source of error (shrinkage from absorption of a large hemorrhage) can easily be eliminated by histological examination. This has, in fact, been done in the present series, 1 tumor which diminished rapidly in size having been omitted from the count because of the presence of a large blood-filled cyst. Nor have there been included a case in which a regressing neoplasm was found to contain only keratin, without living tumor cells; 3 in which diminishing nodules which might have been tumors were composed entirely of connective tissue; 1 in which only blood-filled acini could be found in the section; 1 in which the section contained nothing but necrotic material; and 1 case in which the tumor had decreased too little in size to suggest that it would probably have regressed further. In brief, no case has been included in which actual living carcinoma could not be demonstrated in the section.

BIOLOGY

Race has nothing to do with the regression of spontaneous carcinomata, since mice from four different dealers appear in the present series. Indeed, one of the animals (1481) came from a cancerous stock under cultivation at that time in the Institute, and might therefore have been supposed to be especially vulnerable to malignant disease, for its cancerous heredity was much more concentrated than that of the ordinary mouse.
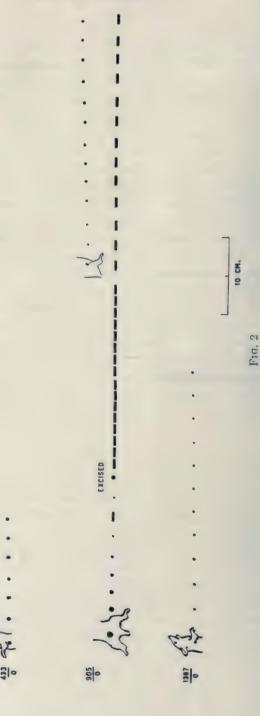
Attention has been paid, also, to the time of year at which recession took place, and this was found entirely without influence, as was to be expected. Spontaneous cure took place at all seasons.

RECEDING SPONTANEOUS MOUSE CARCINOMATA
CHARTED WEEKLY UNTIL THE
DEATH OF THE
MICE

Fig. 1

381

STATIONARY OR FLUCTUATING SPONTANEOUS MOUSE CARCINOMATA
CHARTED WEEKLY UNTIL THE
DEATH OF THE
MICE

Fig. 2

Nor is pregnancy a necessary antecedent of retrogression, for although Miss Slye (3) believes that it retards the growth of spontaneous neoplasms in the mouse, only 1 out of 16 animals in the present series was pregnant.

It does not seem at all likely that any constitutional alteration in the organism underlies spontaneous cure. Haaland, who has already discussed this question, concluded that the evidence which he was able at that time to adduce suggested the importance of local conditions, probably arising within the tumor cells themselves, rather than the intervention of general constitutional changes. This conclusion was drawn because he had observed the disappearance of one spontaneous tumor and the concomitant growth of another in the same mouse.

Further evidence supports his judgment, for in the present group of 13 mice with receding tumors this phenomenon has occurred twice (772 and 1122, fig. 1). Again, the complete recession of a carcinoma is not the result of a general change rendering the animal unsuitable for its immediate recurrence, for this event, also noted by Haaland, took place in mouse 905 (fig. 2); even the few live cells remaining could not be destroyed by the host. Nor does regression prevent the later development of another carcinoma (905, fig. 2, and 1481, fig. 1). In short, a mouse with a receding carcinoma may also bear an actively growing one at the same time; or its organism may permit immediate recurrence of a tumor that has regressed so far as to be no longer palpable; or, finally, a carcinoma may arise some weeks after another one in the same animal has diminished considerably in size or disappeared. The evidence is incontrovertible except in the case of mouse 905 (fig. 2), in which the second tumor remained stationary in size; and it is difficult to escape Haaland's conclusion that the cause for spontaneous cure is to be sought in the tumor, rather than in the organism of the host.

Whether it is to be looked for in the tumor cell itself, however, is another question. It has recently been suggested (4) that the disappearance of transplanted neoplasms may be the result, in part at least, of thrombosis of their veins; but for want of

adequate material it is quite impossible at the present time to extend this conception to the regression of spontaneous new growths of the mouse.

<center>HISTOLOGY</center>

Haaland has studied with great care the microscopic appearance of receding spontaneous carcinomata of the mouse, and there is but little to be added to his description. The rarity of regression is the only reason for presenting additional histological studies. In order that histology and biology may be compared, the morphological data are given separately for each tumor.

$\frac{125}{0}$ A non-hemorrhagic adenocarcinoma. The sclerotic and hyaline stroma described by Haaland is not present. There is no unusual infiltration with small round cells, no necrosis, and there are no "cholesterol slits." A small amount of hemorrhage has occurred in the stroma at the periphery of the growth. The number of mitotic figures is moderate.

$\frac{433}{0}$ A keratinizing carcinoma, with no living tumor cells in the section, complete keratinization having taken place. This tumor may fairly be included in the series, however, because the mouse had a metastasis in the lung with no other neoplasm to account for it. The stroma of the regressing tumor is not sclerotic, there is no unusual round cell infiltration, no necrosis, and there are no "cholesterol slits."

$\frac{514}{0}$ A non-hemorrhagic adenocarcinoma. The stroma is not sclerotic, neither is there any unusual infiltration with small round cells. Hemorrhage and "cholesterol slits" are absent. There is total necrosis of large portions of the acini (fig. 3). Such widespread necrosis as this has not been found in any other growth of the present series, or in Haaland's cases. There is an occasional mitotic figure to be seen, though this tumor was preserved after the animal had died.

$\frac{525}{0}$ Hemorrhagic adenocarcinoma. There is neither sclerosis of the stroma nor unusual round cell infiltration. No "cholesterol slits." Widespread hemorrhage in stroma and parenchyma. Although this tumor was preserved after the death of the animal, it contains a moderate number of mitotic figures.

$\frac{759}{0}$ A non-hemorrhagic adenocarcinoma.   There is but a minimal amount of stroma, which is not sclerotic.   There is no unusual infiltration by small round cells, no hemorrhage, and there are no "cholesterol slits." Small scattered areas of necrosis are present. This tumor contains as many mitotic figures as a growing one, though it was preserved after the animal had died.

Fig. 3

$\frac{772}{0}$ A non-hemorrhagic adenocarcinoma, only half of which is included in the section.   In this available half, the very scanty stroma is neither sclerotic nor unduly infiltrated with small round cells.   There are no "cholesterol slits" and there is no necrosis.   The acini here and there contain a few red blood cells, but there has been no real hemorrhage in either parenchyma or stroma.   Hardly a mitotic figure can be found, though this tumor was removed by operation.

$\dfrac{905}{0}$ The stationary tumor is a non-hemorrhagic adenocarcinoma. There is no sclerosis of the stroma, no unusual round cell infiltration, and no necrosis, nor are "cholesterol slits" present. A few insignificant hemorrhages have occurred in stroma and parenchyma. Decomposition is too far advanced for mitotic figures to be sought.

The growth which receded entirely and afterward recurred and was excised, is a hemorrhagic adenocarcinoma. The stroma is not sclerotic, and there is neither necrosis nor unusual small round cell infiltration. The growth contains a few small hemorrhagic cysts, and there has been some hemorrhage in the stroma. Though this was a growing tumor at the time of extirpation, it contains but a moderate number of mitotic figures.

$\dfrac{950}{0}$ A papillary adenocarcinoma, with dense fibrous stroma that has undergone hyaline change. There is no suggestion that the parenchyma is being destroyed by compression. The stroma (fig. 4) resembles that described by Haaland in receding spontaneous carcinomata, except that the hyaline change is not so pronounced. This is the only tumor among the 16 examined in which extensive sclerosis has occurred. Throughout this dense connective tissue there are occasional small and old hemorrhages, and along one side of the tumor there has occurred rather recently a large hemorrhage. "Cholesterol slits" are present (fig. 4), but there is no necrosis and no unusual round cell infiltration. Many of the acini are dilated, and contain large, pale, swollen, and fatty cells, and where the process has advanced further there is considerable detritus in these spaces, some of which contain red blood cells. This neoplasm, which was removed by operation, contains a fair number of mitotic figures.

$\dfrac{1122}{0}$ A hemorrhagic adenocarcinoma. The stroma in certain portions of the growth is somewhat sclerotic, and has undergone hyaline change here and there. There is no unusual small round cell infiltration, and no necrosis, nor are "cholesterol slits" present. There have been a few insignificant hemorrhages in the stroma. An occasional mitotic figure can be seen, though the growth was preserved after the death of the mouse.

$\dfrac{1295}{0}$ A non-hemorrhagic adenocarcinoma. The stroma is moderately sclerotic in some areas, but not hyaline; it contains small hemor-

rhages. There is no unusual round cell infiltration, no necrosis, and there are no "cholesterol slits." This tumor, which was removed by operation, contains many mitotic figures.

$\frac{1303}{0}$ A hemorrhagic adenocarcinoma. A few areas in the stroma are sclerotic, and the stroma has been the seat of considerable hemor-

FIG. 4

rhage. There is no unusual small round cell infiltration. A few of the acini contain debris and "cholesterol slits." There is very little carcinoma left, and the acinous arrangement of that which remains is often disturbed by hemorrhage. In this tumor, which was preserved after the death of the mouse, there are no mitotic figures to be seen.

$\frac{1387}{0}$ A hemorrhagic adenocarcinoma, containing large cysts filled with the remains of old hemorrhage. The stroma is not sclerotic, and

there is no unusual infiltration with small round cells. No "cholesterol slits" are present. The mouse was so sick that it had to be killed, so that this tumor was preserved fresh. It contains many mitoses, and, like the majority of regressing neoplasms, cannot be distinguished by its histological appearance from a growing one (fig. 5).

Fig. 5

$\frac{1481}{0}$ A hemorrhagic adenocarcinoma, with the remains of old hemorrhages in its cysts. A few of the acini contain detritus and "cholesterol slits." The stroma, into which hemorrhage has occurred, is not sclerotic. There is an area of round cell infiltration, but the cells of acini in the midst of it are dividing freely (fig. 6), and appear to be quite as healthy as those in any other part of the tumor. This growth, which was removed by operation, contains many mitotic figures.

$\frac{1496}{0}$ A hemorrhagic adenocarcinoma. The very scanty stroma shows no suggestion of sclerosis. There is no unusual round cell infiltration except for small areas in the capsule, which are associated with hemorrhage. No necrosis and no "cholesterol slits." In this tumor, which was removed by operation, only one mitotic figure could be found.
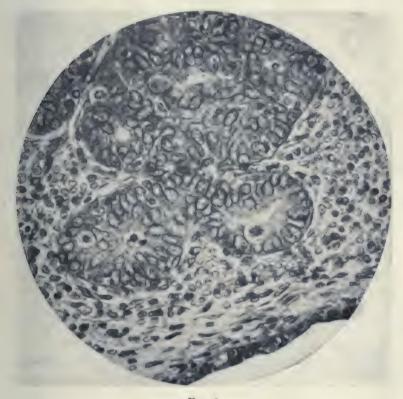
FIG. 6

$\frac{1611}{0}$ A hemorrhagic adenocarcinoma. The stroma is slightly sclerotic in some places, and is the seat of considerable hemorrhage. There is no unusual round cell infiltration, and necrosis and "cholesterol slits" are absent. This growth, which was preserved after the death of the mouse, contains only an occasional division figure.

DISCUSSION

Clearly, none of the histological findings reported above is common to all receding tumors. Furthermore, all can be found in actively proliferating carcinomata, for the first six growing tumors selected at random and examined as controls contained both old and recent hemorrhages into parenchyma and stroma, small round cell infiltration in stroma and capsule, necrotic areas comprising as much as half of the whole growth, and "cholesterol slits." These clefts, which have been described by Mandelbaum (5) and others, occur in various benign conditions also, and are obviously of no consequence in spontaneous cure.

The dense hyaline stroma found in some receding carcinomata appears to be equally without import, since it was found in one of the six growing tumors examined for comparison, though not in such advanced degree as in one of the receding tumors. The absence of widespread sclerosis from 11 out of 16 receding or stationary new growths, and its local distribution in others, appear to deprive it of any significance, and suggest that it is in all probability a mere replacement fibrosis. There seems to be no connection between the rapidity of regression and the amount of sclerosis, for in Haaland's 2 cases recession was very slow, whereas the only tumor of the present series (950) with as advanced sclerosis as Haaland pictures, diminished rapidly in size.

Pronounced infiltration with small round cells appears to be as unimportant as the two preceding phenomena, for it can be found in growing tumors and is often absent from regressing ones. Moreover, when it is present, the cells in its midst may continue in active division. It is sometimes associated with hemorrhage.

Regression may take place in at least three ways, which may or may not be expressions of one underlying cause; on this point no opinion is possible at the present time. In the great majority of cases, the growth (a) recedes without showing any appreciable morphological alteration, so that it cannot be distinguished under the microscope from a growing neoplasm; more rarely, (b) widespread necrosis, or (c) complete keratinization is associated with spontaneous cure.

A most interesting feature about disappearing tumors is the presence in them of a relatively large number of division figures. It is hardly possible to make an accurate estimate, since the number varies considerably in different parts of a tumor and, in all probability, at different times.   Nevertheless, an attempt was made to get some sort of idea regarding the number of mitoses, by counting those in fifty high power fields selected at random from different areas, and delimited for convenience by a small square cut out of cardboard and placed in the ocular.   However, the counts thus obtained are not to be taken too seriously, since it is likely that they are not much more accurate than a general impression would be, expressed in such terms as "many" or "few."

The following numbers of mitotic figures were found in fifty fields from each one of 6 receding neoplasms, which are arranged in the column as nearly as possible in order of their speed of regression, beginning with the most rapid.   Only such tumors as had been preserved perfectly fresh appear in this group.

mitotic figures

Tumor 950.. 21
Tumor 1295.. 38
Tumor 1481.. 50
Tumor 1496.. 1
Tumor 772.. 3
Tumor 125.. 20

A stationary tumor (1387) contained 64 mitotic figures to fifty fields.

It would thus appear that the number of division figures bears no relation to the rapidity of recession, though it must not be forgotten that the data are inadequate for any but tentative conclusions.

The mitoses in these 6 receding new growths are to be compared with the number in 6 growing carcinomata, 3 of which were increasing rapidly in size, while the other 3 were proliferating very slowly.   These, also, had been preserved in fresh condition.

Rapidly growing

	mitotic figures
Tumor 807	86
Tumor 600	28
Tumor 479	11

Slowing growing

	mitotic figures
Tumor 836	43
Tumor 507	38
Tumor 129	12

If these counts be trustworthy, a slowly growing neoplasm may contain more division figures than a rapidly growing one. A certain amount of confidence may, perhaps be placed in them, since the same observation has been made in respect to neoplasms of the human subject by McConnell (6). McConnell found that the number of division figures is not an index of growth speed, for the most slowly proliferating tumors may contain a large number of them, an observation corresponding with the experience of most pathologists.

This seems, in our present ignorance, paradoxical enough. But the situation is still more difficult, for comparison of the figures for receding and growing mouse carcinomata shows that a rapidly disappearing tumor may actually have more mitoses than a vigorously growing one. It has already been pointed out (4) that receding transplanted carcinomata contain relatively large numbers of division figures.

The presence of so many division figures in receding new growths can only mean that they are proliferating actively, and perhaps as vigorously, when regression first sets in, as those that are increasing in size. If so, the difference between advancing and receding neoplasms may be this: In the growing tumor, the parenchyma is augmented faster than it can be destroyed, while in the receding tumor it dies, or is killed, more rapidly than it can be replaced.

Does the cancer cell die, or is it killed? It is difficult to believe that it is destroyed by the host, for in the case of both transplanted and spontaneous neoplasms an animal may bear, at the same time, a growing and a receding tumor. It is not

probable that the disappearing growth has become sensitive to injurious factors in the host which are still inoperative against the advancing one, for regressing transplantable tumors can be propagated with fair success (4), showing that they have not been uniformly injured throughout.

This narrows the search down to the tumor. Does the cancer cell die, or is it killed by something within the tumor? The large number of division figures in many receding spontaneous and transplantable neoplasms, and the transplantability of regressing propagable tumors, do not suggest a generalized fundamental change in the cells of receding carcinomata rendering them incapable of further proliferation. Nevertheless, the possibility that spontaneous cure is the result of localized biological alterations in the parenchyma cannot be eliminated.

Searching now for something within the tumor which destroys the parenchyma, we find that this can hardly be sclerosis, lymphocytic deposits, or hemorrhage, for reasons given above. Only the blood-vessels remain for consideration; but in the spontaneous new growth these can be neither implicated nor dismissed, as has already been explained, though in the case of the transplanted neoplasm there is a certain amount of evidence to suggest that thrombosis of the veins may be one of the factors responsible for the disappearance of a tumor (4).

CONCLUSIONS

No histological feature has yet been found common to receding carcinomata and absent from growing ones; hemorrhage, necrosis, small round cell infiltration, "cholesterol slits," and sclerosis of the stroma occur in both receding and advancing tumors.

A retrogressing carcinoma may be indistinguishable, under the microscope, from one that is increasing in size, or it may be totally keratinized or largely necrotic.

A stationary or receding carcinoma may contain as many mitotic figures as one that is increasing rapidly in size.

The regression of a spontaneous carcinoma in the mouse is probably not brought about by any constitutional alteration in the bearer, because one tumor may recede while another in the

same animal is rapidly growing; furthermore, complete regression does not render the mouse unsuitable for recurrence within a week, nor does it prevent the subsequent development of another carcinoma.

The factor responsible for spontaneous cure appears to reside neither in the stroma nor in the parenchyma of the tumor, though the latter cannot be entirely eliminated. By exclusion, only the blood-vessels remain, but it cannot be shown from the material here discussed that vascular changes underlie spontaneous cure.

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THE NUCLEO-CYTOPLASMIC RATIO AND CANCER

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The problem of malignant tumors is one of those which investigators the world over have tried vainly to solve during the past ten years. There is no similar subject in which the bibliography is so bulky and the actual results so scanty and unsatisfying. The final solution still appears as distant as it was forty years ago. However, interest in the question is very great, because of its biological character. As yet inexplicable biological phenomena lie at the foundation of the problem; as for example, the cause of the unrestrained growth of the cancer cell. The biological importance of this phase of the question is evident to every investigator with the exception of those infrequent partisans who still cling blindly to the parasitic theory. But the strictly biological researches which have been made on the cancer question are the least complete of any mode of approach, most of the attacks being directed from the point of view of pathology or of experimental medicine. The nature of the biological and physiological qualities of the cancer cell is the first thing which strikes the biologist. Boveri, who particularly devoted himself to this side of the cancer problem says, and we must recognize he was right, that the tumor problem is a cell problem, which means in other words that the solution of the problem of the malignant tumor is intimately connected with the question of the tumor cell.

According to Boveri, a malignant tumor is a consequence of the appearance in healthy tissue of foreign cells in a diseased condition, whose vital activities are especially vivid, and which are distinguished by their extreme capacity for growth. Boveri inquires as to the site of this defect in the cell structure, and he believes that it is not in the protoplasm of the cells, but in their

nuclei. Most of the authors who have studied the cytology of malignant tumors cite facts which show that the normal relationship existing between the nucleus and the cell body is lost in cancer tissue. In addition to the classical researches of von Hansemann, Arnold, Boveri, and others, devoted to the general cytological study of neoplastic tissue, there are also special studies of the relationship between the nuclei and the cells of cancerous tissue (Heiberg, Aichel). As I have already shown in 1912, when reporting observations concerning cultures of cancer tissue in vitro, there is a curious transformation of the nucleo-cytoplasmic relationship in the tissues of malignant tumors, but it is only in the past year at Brussels that owing to the kindness of Professor Dustin, who has put at my disposal his very valuable collection of sections of malignant tumors, I have been able to complete my work, which illuminates in detail this particular phase of the question.

MATERIAL AND TECHNIQUE

I have limited myself in my investigations to the study of carcinoma, for technical reasons principally, as it is more difficult to measure the cells of a sarcoma and make accurate statements concerning the morphological relationships so determined. The collection of epitheliomata of Professor Dustin is particularly rich, and I have also had the opportunity to study not only human cancer, but also that of mice, in several forms and phases of development. The study of the nucleo-protoplasmic ratio[1] presents considerable difficulty. At times the increase in the size of the nucleus is so slight that it may completely escape the most careful observation. On the other hand, taking cells at random for measurement often leads to very doubtful results, and the conclusions drawn therefrom may be entirely incorrect. Hertwig and his co-workers advise in order to avoid such errors, that a very large number of measurements be made, and this rule has been followed in my own work. The methods of measurement and the averages derived from such measures, in order to

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[1] This will be abbreviated hereafter as the N:P ratio.

establish a ratio between the nucleus and the cell body, have differed with different investigators. In the study of the Protozoa and Infusoria, where the dimensions of the body are easily measured in three directions, the ordinary formula for determination of volume is applicable (Hartmann, Popoff, Koehler, Jörgensen, Chambers, Levi). The same formula has been applied to measuring embryonal cells. In this instance the volume of the nucleus as well as the volume of the cell is calculated either by the well known formula for the volume of a sphere $4\pi r^3/3$ if the shape of the nucleus and the cell is spherical or that for an ellipsoid $V = 4\pi abc/3$ if the nucleus and cell have that form, where a, b, and c are the semiaxes.

The following formula, then, represents the nucleo-protoplasmic volume ratio considering both spherical $\dfrac{4\pi r^3/3}{4\pi R^3/3}$ where r is the radius of the nucleus and R that of the cell.

In order to establish the relationship between the nucleus and the cell, however, it is not always possible or necessary to obtain the volume of each. In studying very cellular tissues, especially where the cells are somewhat compressed, it is sufficient to calculate the relation between the surfaces, using the formula $\dfrac{\pi\, ab}{4}$ where a and b are the two diameters. In many instances, especially in mouse cancer, where the form of the cells approaches the rectangular, it is sufficient to multiply a and b in the previous formula, as employed by Berezowski. In my own measurements I have always used the ratio of the surfaces. .Finally the method of fixation of the material plays a certain but insignificant rôle, and scarcely counts in the results. A great variety of fixatives has been employed, using those which gave the best results. The preferable stain is hematoxylin.

THEORIES OF THE NUCLEO-PROTOPLASMIC RATIO

The multiplication of unicellular organisms and of tissue cells is governed by laws that are very complicated and still but little known. The growth of cells, their division, and their death depend upon many factors, some of which are favorable

to the rejuvenation of the cells, while others, on the contrary, lead to their destruction. The problem of cell age and immortality, using the Protozoa as material, is intimately correlated with the relation between the nucleus and the cell body. The problem of the malignant cell and of its growth and its capacity to multiply is also closely connected with the problem of the aging of the cell. Hertwig and his pupils have approached the question from the cytological aspect, studying the transformations which are correlated with the depression, death, and rejuvenation of the cell. These investigations have led to the hypothesis of the vital importance of the ratio between the nucleus and the cell protoplasm for the vital activities of the unicellular organism, since the ratio of the nucleus and the protoplasm has definite values under certain given conditions (Hertwig.) He states:

As in all physical and chemical processes, so also in life processes a certain mass relationship between the active portions must exist, and so also with the life processes of the cell certain definite mass relationships between the protoplasm and the nuclear substance exist, a relationship to which I have given the name nucleo-protoplasmic ratio (R. Hertwig, p. 4).

It is also true

that the N:P ratio is not only an important factor in the cell life, but also a factor which can be exactly determined (Hertwig, p. 8).

This relationship is intimately bound up with the question of the life and growth of the cell, for the growth of the cell is dependent upon assimilation; assimilation is dependent upon food and the use of the same for the construction of living material, and, as we have seen, only under the influence of the nucleus. Our question therefore can be expressed as follows: In what way does the nucleus play a part in this most important process, and what changes does its structure undergo in the course of its activity during the process of assimilation?

The study of the N:P relationship and the establishment of the fundamental principles connected with it permits a better understanding of the processes of division, for "division is the condition of the most energetic activity of the cell in which forces which remain quiet during growth suddenly come into activity" (Hertwig, p. 19).

Gerassimoff, who commenced his investigations on the N:P ratio about the same time as Hertwig, also determined for Spirogyra that the growth and the division of the cells depend upon the relationship between the nucleus and the cell body. He has found, for example, that the cells with nuclei which have twice the volume of the normal do well under favorable conditions but do not divide when conditions are unfavorable, and that division commences only when the volume of cell protoplasm is increased twice the normal amount. Thus there seems to be some important factor in the N:P relation which suggests that it is an indispensable condition for the vitality of the cell, and that the disturbance of this ratio is intimately connected with the phenomena of depression. Maupas, R. Hertwig, Prowazek, and Jennings have described these phenomena in the Protozoa. Popoff has stated that in the stage of depression of the Infusoria the macronucleus is considerably enlarged and occasionally vacuolated. In the multicellular organisms, Harry Marcus observed the same phenomenon in the later stages of development of the thymus in the Gymnophiores. Other authors have described similar phenomena, e.g., Frischhols, in hydra, and Reichenow, in tadpoles.

Gruber, who studied the N:P ratio in ameba, believes in the importance of the normal relationship for the vitality of the cell. If a portion of the cell body was removed by operation, he found that a diminution in the size of the nucleus took place. Thus experiments directed the discovery of the causes which disturb the N:P relationship; the hypertrophy of the nucleus and the degeneration of cells are interesting not alone as an attempt to explain the multiplication and the vitality of cells, but may also concern the problem of tumor malignancy.

Temperature exerts a very great influence on the relation which exists between the volume of the nucleus and that of the cell. Robert Hertwig first established this fact, and many others have confirmed it. Koehler, for example, has confirmed Hertwig's opinion from measurements made on the nucleus of Strongylocentrotus, and the relative size of the nucleus is found to be less at a high temperature as compared to its size at a low.

Koehler has furnished the coefficient which shows the influence of temperature on the relationship of the volume of the nucleus to that of the cell. At high temperatures the N:P ratio may be 1.0, while at low the ratio may be 1.945. The studies of Rautmann on Paramecia confirm the influence of temperature as have also the studies of O. Hartmann on Cladocera and Ceratium. Hartmann believes that the influence of temperature on the N:P ratio should be considered as a factor determining metabolism in the cell and its vital activity. This theory explains some observations made by Papanicolau, who believes that cold and starvation are the active factors in the production of sexual multiplication, while ample food and heat facilitate the parthenogenetic type. Among the Protozoa, as well as the Daphnids, asexual multiplication can be considered as having a greater vital intensity than sexual multiplication. Among the protozoa, at least, it should be considered as a result of depression. Among other natural factors which influence the N:P ratio, nutrition also plays a very important rôle. Kazantsef was one of the first to study in paramecia the transformation of the nucleus and the cellular protoplasm induced by starvation. Similar studies have been made by Sosnovsky and Khainsky. The latter examined very carefully the effects of hunger in paramecia and observed two periods during the fast; the first characterized by diminution of volume and disappearance of the nutritive corpuscles; the second by death of the cell and breaking up of the very much enlarged nucleus.

Lipska examined the effect of starvation on Infusoria and showed that they are able to go without food for days, occasionally as long as three weeks. Among the morphological alterations noted was that the micro-nucleus increased in size and divided into two portions, becoming separated from the macro-nucleus. All of these observations show that hunger promotes abnormal augmentation of the size of the nucleus, vacuolation, and ultimate degeneration. I have shown however (Sokoloff, IV) that these phenomena do not appear with short fasts, but only after prolonged abstention from food. On the other hand, Robert Hertwig noted in his studies on the Actinosphaerium

that "one of the causes of depression lay in excessive growth of the nucleus in relationship to the protoplasm, which might be caused by over-feeding. The tendency to depression so induced accentuates the depressive effect of hunger." Multicellular organisms show the same result of fasting. Detailed studies of Marie Krahelsky have shown the alterations in the N:P ratio as seen in the cells of the albumin gland of Helix. As the nucleus increases the volume of the protoplasm diminishes, while prolonged fasting decreases also the absolute surface of the nucleus which, however, remains relatively increased in proportion to the area of the protoplasm as the following figures demonstrate.

1. Normal gland of Helix. Surface of nucleus 2.19; surface of protoplasm 48.89; nucleo-protoplasmic ratio 0.045.

2. In Helix which has fasted for ten days, the surface of the nucleus is 2.72; surface of the protoplasm is 12.16; nucleo-protoplasmic ratio 0.223.

After a prolonged fast the N:P ratio becomes still larger.

Morgulis, in his general review on the influence of over-nutrition and fasting on the cells of multicellular organisms, attempts to explain the changing ratio by assuming the loss of water from the organism. The protoplasm of the intestinal epithelium after a month of fasting loses 62.3 per cent of its volume; the nucleus loses much less, hence the alteration in the ratio.

It is well known that certain chemical substances alter the N:P ratio, including enlargement of the nucleus in proportion to the concentration of the solution and the duration of the experiment, thus giving a more or less complete picture of the cellular depression. Popoff (III) experimented with water charged with carbon dioxide; Boris Sokoloff (V) with a series of alkaline salts, Hainsky with oxygen. All showed an alteration of the ratio as a result of the changes in the external medium of the cell. Similar experiments were made by Godlewski, Jr., who observed that sea-urchin eggs in sea water charged with CO_2 undergo nuclear division without separation of the cytoplasm, thus obtaining multinuclear cells resembling somewhat the giant cells seen in cancer tissue. In several instances the chromatic

substance, which was extremely enlarged, became sub-divided in the same cell into several nuclei. Berezowski (III) showed that castration in white mice increases the superficial area of the cell. Thus a number of different causes can induce alteration in the ratio. These are closely connected with hypertrophy of the nucleus, an indication of depression of the vitality of the cell, though the exact nature of this depression is as yet unknown. However, it can be stated that in the normal cell the relation between the nucleus and cytoplasm is constant, while under unfavorable conditions this ratio is altered. The nucleus enlarges, the organism of the cell loses its vitality, and as a result of the depression which follows becomes senile and dies. But O. Hartmann justly calls attention to the fact that the term "depression," in so far as it is manifested by increase in the size of the nucleus, may indicate very different things and, similarly, a high or a low protoplasmic ratio may possess very different physiological parameters.

We have already seen that the hypertrophy of the nucleus follows changes of temperature. However, in this last instance the decrease in the size of the nucleus is very often only a physiological manifestation and not a cytological evidence of depression. I have shown in my paper (II and III) on regeneration of Protozoa that starvation of long duration, even when accompanied by a considerable hypertrophy of the nucleus, does not necessarily lead to the death of the cell, but on the contrary may be one of the phenomena of its greater vitality. It is therefore more rational to use the term "depression" in connection with alteration of the N:P ratio only when this is accompanied by other marked evidence of cytological alteration (Popoff); that is, when the nucleus enlarges and becomes multilobular or vacuolated, or the micro-nuclei are increased in number. Hertwig's pupils have shown that in embryonic tissues the N:P ratio is not the same as in the adult animal, the nucleus being much larger in relation to the protoplasm in embryonal tissue. Jörgensen has shown in Patella, for example, that the ratio is 1:1.5 in the egg; in the later stages the nucleus diminishes relatively, and the ratio becomes 1:6, and in the adult animal may finally

reach 1:12; in other words, the relative size of the nucleus diminishes with age. This relative diminution has often been mentioned, for example, by B. Sokoloff (I) in his studies of Gregarina cystobia intestinalis (N. sp.). In the early stages the nucleus is relatively large, so that it almost fills the cell; the ratio is 1:6; in the adult animal, however, this ratio changes and the coefficient does not excede 1:30. A detailed study of this question was made by Siedlecki, working on the Gregarina Lankesteria ascidiae. In the early stages, soon after the parasite has penetrated into the cell, little alteration is noted in the ratio. As soon as growth commences the nucleus is preponderant while preponderance of the cell protoplasm is only noted at a later period. Similar facts have been reported by A. Berezowski in white mice. In animals aged ten days the ratio is 1:4.9; in these of four months the ratio is 1:6.6. Jörgensen also calls attention to the fact that the nucleus and the protoplasm do not increase at the same rate during the growth of the egg, young eggs having nuclei relatively larger than those which are older. Gonolin, on the other hand, studying Gastropods, did not find a sensible difference between the ratios in the muscular and connective tissues in young and old animals, but only in the epithelial structures.

The work of Eycleschymer on the muscle cells of Necturus, and that of Schiefferdecker on the human embryo and the muscles of the frog, have shown that the size of the nucleus in embryonal tissue is much greater than in the adult.

The work of Lams remains isolated among all these investigations; he states that in Ario the ratio remains constant at 1:1.6 during the development of the animal.

But this is only one exception which does not, therefore, break down the general rule. It is evident, however, that in the young cell the growth of the nucleus is not a sign of depression as is the case with functional hypertrophy, when the nucleus becomes frequently lobulated, alters its form, and shows vacuolization. Thus it must be concluded, making allowance for facts such as the influence of temperature and age, that augmentation in the size of the nucleus is not always a sign of cellular

depression and approaching death, but on the contrary that other evidences of degeneration must also be present, for the embryonal cell, while its nucleus is relatively large, has a great growth activity.

There is another aspect of the N:P theory which complicates somewhat the fundamental principles, and this is the relation between the size of the nucleus and the chromosomes. It is important to determine whether it is the number or the volume of the chromosomes which varies in the cell, and also what relationship exists between the quantity of the chromosomes and the enlargement or diminution of the nucleus. The classical investigations of Němec on the relationship between the number of the chromosomes and the size of the nucleus in Malva crispa, and the studies of Schellenberg, Gregory, and Gonelin, show considerable defects in this aspect of the theory of the N:P relationship. Despite most careful and minute cytological investigations a wholly satisfactory answer cannot be given to the questions which have been stated above. These facts give rise to a certain opposition manifested by some investigators to the principle of the theory of the N:P relationship, and doubt as to the utility of this ratio in scientific work has been cast, for example, by Gurwitsch (page 37), who holds that the Hertwig principle is only a special case of cell growth and has no application in any other conditions. This criticism, however, is not fatal. Especially for our problem of the abnormal growth of the cells of malignant tumors, the theory of the N:P relationship is not only a good working hypothesis but also a theory which suggests certain lines of investigation, and at times more or less valuable results.

THE NUCLEO-PROTOPLASMIC RELATIONSHIP AND THE MALIGNANT CELL

After the survey of the field given above, we now turn to the application of the ratio to the problem of malignant tumors. As we have already said, the causes of the vital activity of malignant cells is the fundamental aspect of the problem which interests us; what are the facts of this ratio as applied to tumor cells? Even a very superficial study of cancer tissue shows pronounced

cellular alterations; marked nuclear hypertrophy is an unquestioned fact, generally acknowledged by all cytologists who have studied such tissues, but this hypertrophy of the nucleus is not the same in different cells in the same tumor. There are two principal types of carcinoma cells and of hypertrophic nuclei which can be distinguished. In one the nucleus is but slightly enlarged and does not show any evidence of degeneration (figs. 1 to 4). Cells of the second type are seen most often in degenerating tumors (figs. 5 to 8). When methods of fixation adapted to the preservation of chondriosomes are employed (Meves, Sjöval), mitochondria can be demonstrated in the cells. They have the form of short rods and of round granules, as have been described by other authors (Favre and Regaud, Veratti, Savagnore). The mitochondria are distributed evenly throughout the cell body, but when the decrease in the size of the nucleus begins and degeneration sets in, they lose their regular character and form voluminous granules. It can also be demonstrated that some cells, apparently those which are the youngest, and also some which are very old (figs. 9 and 10), do not contain mitochondria. We do not propose to take up here the very complicated cytological alterations which are found in tumor cells, but rather to study the nucleo-protoplasmic ratio in order to determine whether a tumor cell should be considered as a rejuvenating one, or one which is dying, or in a degenerative stage. In answer to this question I made a large number of determinations of the ratio in tumor tissue, directing my investigation both to tumors in mice and a number of types of human carcinoma.

A. Carcinoma of the white mouse

In order to investigate mouse carcinoma tumors were studied which Professor Dustin kindly placed at my disposal, together with several collections of sections already completed. The histological technique has already been mentioned. In measuring the formula of Berezowski has been employed. The comparison of my work with that of Berezowski is important because the latter made a careful study of the N : P ratio in the tissues of white mice, thus furnishing a standard of comparison with malignant

tissues. Berezowski made his measurements in the following manner: The length and breadth of each cell was measured, and also the largest and smallest diameters of the nucleus. Fifty such measurements were made in each case, and the average results were computed. As only cells of approximately rectangular form were measured, the area may be obtained by multiplying the average length of the cell, expressed in micra, by their average width. The form of the nucleus is elliptical, and in consequence the nuclear area can be computed by the formula $\frac{\pi}{4} ab$ in which a is the largest diameter of the nucleus, and b the smallest (Berezowski, p. 380). I have followed the above method, with the exception that I used the formula instead of simply multiplying the diameters of the cell, and also increased the number of measurements to 100 to diminish errors of sampling. Berezowski made his measurements chiefly on epithelial tissues of newly born, young, and adult white mice. The fact that he did not measure the cells of the embryo is regrettable, for the result would have been interesting. He showed that cells of very young animals, which ontogenetically are related to embryonal cells, possess nuclei larger than those of adult animals, where growth has been completed, as the following figures prove: In mice ten days old, the ratio is 1:4.9; in those of two months 1:6.1; and in those of four months, 1:6.6. Berezowski believes that the change in ratio is due to relative diminution with age of the nucleus as compared to the cell. At maturity the ratio decreases about $1\frac{1}{2}$ times. My own measurements, made from ten different carcinomata of the white mouse, furnish figures which show the close relationship between cancer tissue and embryonal tissue. The figures are given in table 1. The results so tabulated furnish average coefficients of the relation of the nucleus to the cell in epithelial tumors of the mouse. This coefficient is 1:4.3; in other words, the nucleus is on the average larger than that which Berezowski found in the epithelial tissue of newborn white mice, and approaches the figures for embryonic tissue. In the measurements of these tumors taken separately we find a great variation in the coefficient; for example, in 10

per cent of the cells the coefficient is 1:2.5; 15 per cent of the cells have a coefficient 1:5.8. In another series, on the other hand, for example, that marked M, in very few of the cells (scarcely more than 2 per cent) does the ratio exceed 1:2.5; but, as will be seen later, these variations are very much greater in man. To find a reason for the great number of cells having a high coefficient measurements were made on tumors at different stages of growth taking for example, a tumor of eight days, another of fifteen days, and so forth.

TABLE 1

	NUMBER	AREA OF NUCLEUS	AREA OF CELL	RATIO
		$\mu2$	$\mu2$	
Mouse a[1]............................	1	18.2	88.2	4.5
Mouse B.............................	2	21.0	81.2	4.2
Mouse Z.............................	3	19.3	78.1	4.1
Mouse no. xvii......................	4	20.0	88.4	4.4
Metastases..........................	5	18.1	83.2	4.6
Mouse M............................	6	18.5	83.1	4.6
Mouse M............................	7	17.2	84.8	5
Mouse N[1]..........................	8	22.2	88.1	4
Mouse b.............................	9	20.1	84.3	4.2
Mouse b[1]..........................	10	21.2	80.5	3.8

TABLE 2

AGE OF TUMOR	AREA OF NUCLEUS	AREA OF CELL	RATIO
days	$\mu2$	$\mu2$	
8	18.9	85.6	4.4
15	21.2	94.8	4.5
20	22.6	92.6	4.1

In the older and larger tumors there are found a greater proportion of cells with extremely hypertrophied nuclei. In such case there is an accompanying degeneration which is well marked. These facts explain to a certain degree the cause of the variation of the coefficient. A comparison of the N:P ratio in cancer tissue and healthy tissue of the same animal furnishes a comparison between the cancer cell, the embryonal cell, and the young cell; but it is necessary to make exceptions for those cells of cancer tissue in which the nucleus is very much hypertrophied,

with all the marks of degeneration. My own results, like those of Schiefferdecker and Berezowski, do not completely agree with those of Heiberg, published in 1908 and 1910. This writer measured the size of the nuclei of cancer cells in mice and found the nuclear increase very pronounced, but he did not believe it possible to attribute embryonal characters to cancer cells. It is his opinion that the nucleus is smaller in young animals than in adult mice. On the other hand, his work published in 1910, devoted to the measurements of the Jensen mouse carcinoma, confirms the considerable increase of the size of the ratio in cancer cells. The coefficient which he obtained approximates very closely that given by my measurements.

B. *Human carcinoma*

Human carcinoma is the other object of my investigations. Professor Dustin put at my disposal about forty preparations, and I was also able to examine a number of carcinomata from the Polyclinic of the University of Brussels, through the kindness of Professor Mayer. The sections included about fifty examples of cancer of the larynx, tongue, cheek, lip, eyelid, nose, ear, skin, and so forth. With the variety of the forms studied it is possible to draw some general conclusions from the material. It was evident from the first that greater variations are present in human carcinoma than in that of mice. There is quite regularly a relative abundance of cells having hypertrophic nuclei, with marked evidence of degeneration. In other tumors the coefficients of the nucleo-protoplasmic ratio were relatively high in most of the cells, but there was complete absence of any evidences of degeneration. This complicates the question. It was necessary to augment the number of measurements, but the ultimate results are the same as that obtained from mouse cancer. Like the latter, human carcinomata also have two types of cells; some with nuclei slightly enlarged, behaving like active cells, others with hypertrophied nuclei, often multilobular and in a state of disintegration. The ratio of these degenerated types is always very high. It amounts at times to 1:1.5 to 1.3. The proportion of these cells in the tissues varies considerably.

There are some sections in which every eighth or tenth cell shows this type of greatly enlarged nucleus. In other sections large nuclei were the exception, appearing only in about 1 to 50 cells. The physiological qualities of these two varieties of cells, and their cytological alteration, have not been studied in this paper, but will occupy a later report. I will only say that cells showing those nuclear changes to which the terms depressed or degenerated have been applied, are dying cells, and they are not those which play any part in the growth activity of the malignant tumor. In comparing human and mouse carcinoma the most striking thing is the great variation of the coefficient in human tumors as compared with those of the mouse. Table 3 shows that the ratio is often higher in epithelioma of the skin than in other types of epithelioma. This is probably due to difference in age of the tumor, but even in the same tumor the same variations in coefficient may be found, far greater than those in mouse tumors. It is also observable that not only may the cells of the same tumor vary greatly, but also that their coefficients may be one and a half to two times those of other cells of the same growth. In an epithelioma of the tongue, for example, there were found cells with a coefficient of 1:4 or 1:4.2, side by side with those having coefficients of 1:2.2, 1:2.3. In spite of these great variations between the cells, we have been able to obtain an average coefficient for most of the tumors examined. The limit of variation of this coefficient lies between 1:3 and 1:5. The average of all of our measurements is 1:4.08. Using these figures, we are able to establish a parallel between cancer tissue on the one side, and normal tissues of the embryo and the adult on the other. It must not be forgotten, however, that there normally exists a great difference between embryonal and adult cells in respect to their nuclear ratio, the ratio being much higher in embryonic than in adult tissue, a fact already noted. "The nuclei of muscle cells from embryos of the fourth or fifth month are considerably greater not only than those seen in newborn, but also in the adult (Schiefferdecker, p. 418). This author gives the volume of the nucleus of the human embryo as 168.64, of the newborn as 68.00, and of the adult female at twenty-nine

TABLE 3

	NUMBER	AREA OF NUCLEUS	AREA OF CELLS	RATIO
		$\mu2$	$\mu2$	
Epithelioma of larynx..................	1	82	263	1:3.2
Epithelioma of larynx..................	2	72	214	1:3
Epithelioma of larynx..................	3	81	285	1:3.5
Epithelioma of larynx X...............	4	61	234	1:3.8
Epithelioma of larynx Y...............	5	73	288	1:3.9
Epithelioma of larynx Z...............	6	84	270	1:3.2
Epithelioma of tonsil..................	1	68	280	1:4.1
Epithelioma of tonsil..................	2	47	220	1:4.9
Carcinoma of tongue..................	1	96	335	1:3.5
Carcinoma of tongue..................	2	95	350	1:3.7
Carcinoma of tongue..................	3	66	280	1:4.2
Carcinoma of tongue..................	4	58	260	1:4.5
Epithelioma metastatic in nodes........	1	29	180	1:6.2
Epithelioma metastatic in nodes........	2	30	165	1:5.5
Epithelioma metastatic in nodes........	3	40	210	1:5.2
Epithelioma squamous cell.............	1	66	257	1:3.9
Epithelioma squamous cell.............	2	69	283	1:4.1
Epithelioma squamous cell.............	3	92	305	1:3.3
Epithelioma squamous cell.............	4	80	290	1:3.6
Epithelioma squamous cell.............	5	65	245	1:3.8
Epithelioma squamous cell.............	6	69	250	1:3.7
Epithelioma squamous cell.............	7	86	276	1:3.2
Epithelioma of vocal cords............	1	53	180	1:3.4
Epithelioma of vocal cords............	2	84	243	1:2.9
Carcinoma of maxilla..................	1	114	380	1:3.3
Carcinoma of maxilla..................	2	91	330	1:3.6
Epithelioma of cheek..................	1	44	187	1:4.2
Epithelioma of cheek..................	2	63	220	1:3.5
Epithelioma of cheek..................	3	76	273	1:3.6
Epithelioma of uvula..................		61	202	1:3.3
Epithelioma of lip.....................	1	79	420	1:5.3
Epithelioma of lip.....................	2	73	350	1:4.8
Epithelioma of lip.....................	3	85	444	1:5.2
Epithelioma of ear....................	1	28	150	1:5.2
Epithelioma of ear....................	2	43	192	1:4.4
Epithelioma of ear....................	3	29	140	1:4.8
Cancer of the skin....................	1	66	270	1:4.1
Cancer of the skin....................	2	43	216	1:5
Cancer of the skin....................	3	33	176	1:5.3
Cancer of the skin....................	4	44	194	1:4.4
Epithelioma of nose...................	1	59	214	1:3.5
Epithelioma of nose...................	2	49	185	1:3.8
Epithelioma of nose...................	3	46	190	1:3.9

years as 71.37, and believes that even greater variation may be found in other tissues.   I have examined several human embryos varying from three to four months of age, and found that the average coefficient of 300 measurements of epithelial cells was 1:3.9.   For each embryo separately I obtained 1:3.6, 1:4, and 1:4.2.   Comparing these results with those which I obtained for human cancer, and with the average coefficient for the epithelium of an adult male, which is 1:6, we are able to see the parallelism which exists between all these figures.   The similarity of the ratio in cancer cells and in embryonic tissue cells gives weight to the view that the N:P ratio and the size of the nucleus is the same in the cancer cell and in the normal epithelial cell of the human embryo.

It is true that this idea is not new.   Many other authors, for example Boveri and Cornil, speak of the embryonal character of cancer cells, and find in this assumption the explanation of their great vitality.   My somewhat limited researches on the nucleo-protoplasmic ratio theory confirm in an indirect fashion what other investigators have found in studies directed toward other problems.   I believe, however, that the fact is established that the source of energy which is manifested in the malignant tumor and in embryonal tissue, at least from a biological point of view, lies in the increase in size of the nucleus and in the very high coefficient of the N:P ratio.

CONCLUSIONS

1. The theory of the nucleo-protoplasmic relation assumes that the vitality of the cell depends on the ratio which exists between the nucleus and the cellular cytoplasm.

2. The constant which defines this relationship varies within the limits of the life of the cell, with temperature, and with the age.

3. Immature cells, and especially embryonic cells, have a high nucleo-protoplasmic ratio and possess coincidently a much greater vital activity.

4. In the tissues of malignant tumors two types of cells are present: one, in which the nucleus is much enlarged, possesses

the character of a depressed cell; the other, with nuclei not especially enlarged, exhibits no evidence of any such depression.

5. Cells of the latter type, that is, cells showing no evidence of depression, whether in mouse carcinoma or in various types of human carcinoma have a nucleo-protoplasmic ratio approaching that of embryonal cells.

6. The results of investigations upon the nucleo-protoplasmic ratio strengthens the assumption that malignant tumors are related in their biological qualities to embryonic cells; that the cells of malignant tumors are in some essentials closely related to embryonic cells, which they resemble in their extraordinary vital activity.

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EXPLANATION OF FIGURES

Fig. 1. Epithelioma of the larynx. The nucleus, N, has the shape of a flattened oval; the mitochondria, M, are disseminated evenly throughout the cell body. Fixation by Sjövall's method. Iron hematoxylin stain.

Fig. 2. Epithelioma of the larynx. The nucleus, N, is large; the mitochondria, M, are irregularly disseminated. Fixation by Sjövall's method. Iron hematoxylin stain.

Fig. 3. Carcinoma of the breast. The nucleus, N. Mitochondria, M. Fixation and stain, Meves.

Fig. 4. Carcinoma of the breast. The nucleus, N, is increased in size. Fixation and staining, Meves.

Fig 5. Epithelioma of the larynx. The cell is degenerated; the nucleus, N, occupies the larger portion of the cell. The mitochondria, M, are in the form of a few coarse granules. Fixation, Sjövall. Stain, iron hematoxylin.

Fig. 6. Epithelioma of the larynx. The cell is degenerated. The nucleus fills almost the whole cell. The mitochondria are only a few voluminous granules. Fixation, Sjövall. Stain, iron hematoxylin.
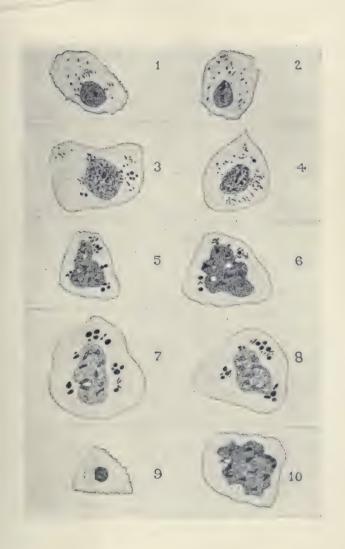
Fig. 7. Carcinoma of the breast. The cell is degenerated. The nucleus is considerably enlarged with diffuse contours. Fixation and stain, Meves.

Fig. 8. Carcinoma of the breast. The cell is degenerated. The nucleus, N, is very much enlarged. Fixation, Meves.

Fig. 9. Epithelioma of the larynx. A very young cell. The nucleus, N, is very small. Mitochondria are not present. Fixation, Sjövall. Stain, iron hematoxylin.

Fig. 10. Epithelioma of the larynx. The cell is very much degenerated. The nucleus is disintegrated. Fixation, Sjövall. Stain, iron hematoxylin.

THE SALT CONTENT OF MALIGNANT TISSUES

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The experiments reported in this communication were originally undertaken in an effort to determine the location within the cell of various chemical substances, and if possible to establish the quantitative variations of these compounds in different types of benign and malignant cells. In the initial experiments, microchemical examinations of the following tissues were made: growing, and spontaneously receding, transplanted Flexner-Jobling rat carcinoma; growing and spontaneously receding transplanted Jensen rat sarcoma; growing rat sarcoma Crocker Institute No. 8: spontaneous sarcoma of the rat liver (Bullock and Curtis stock); adult rat testes; adult rat liver; skin of the rat embryo at term; healing wounds of the rat integument. There were in these various tissues: cells that were malignant and arising in the present host; malignant, but not arising in the present host; malignant, but being destroyed by the present host; actively proliferating but not malignant; and quiescent. Thus all stages of proliferative cell life were represented. The tissues were prepared by suitable staining procedures and examined for chlorides, phosphates, sulphates, iodine, potassium, sodium, calcium, organic and inorganic iron, copper, intracellular ferments, glycogen, oleic acid, olein, cholesterin, neutral fats, fatty acids, and lipoids.

Since the original papers describing the staining methods used may not be available to all, the various procedures are set forth in detail.

Potassium. (Macallum: Die Methoden der biologischen Mikroanalyse, Abderhalden, Handbuch der Biochemischen Arbeitsmethoden,

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vol. 5, sec. 2, p. 1099–1147.) Fresh tissue is frozen and cut and imme-
diately placed in the ice-cold reagent for from thirty to ninety minutes.
The reagent is prepared by dissolving cobalt nitrate 20, and sodium
nitrite c.p. 35, in dilute acetic acid (glacial acetic acid 10, distilled
water to 65) 75. After standing several hours the mixture is filtered
and made up to 100. The section is then washed six or seven times
within twenty minutes with ice-cold distilled water. The potassium
appears as an orange red precipitate, which becomes black when the
section is flooded and mounted in glycerin-ammonium sulphide (equal
parts of glycerin and of ammonia water through which hydrogen sul-
phide has been passed until the odor of ammonia is replaced by that
of hydrogen sulphide).

Chlorides. (Macallum, ibid.) Frozen sections of unfixed tissue are
placed in a mixture consisting of 25 cc. of 60 per cent nitric acid added
to a liter of tenth normal silver nitrate, made in distilled water free of
chlorine and ammonia; the tissue is left in this for thirty minutes,
exposed to the sunlight. The reagent is then poured off and the section
mounted in glycerin. The chlorides appear as brown or brownish
black granules or lines.

Sulphates. (Macallum, ibid.) Frozen sections of the unfixed tissue
are placed in a tenth normal lead acetate solution for fifteen minutes.
They are then washed thoroughly in distilled water and placed in a
tenth normal nitric acid for from two to five minutes; after washing again
they are mounted in ammonium sulphide glycerin (see potassium). The
sulphates appear as black or brown granules or lines.

Phosphates. (Macallum, ibid.) Fresh tissue cut in frozen section is
placed for from three to thirty minutes in the reagent described below,
then washed in distilled water and mounted in glycerin.

Solution I. Molybdic acid, c.p. 1, stronger ammonia water (sp. gr.
0.88) 4, to which are slowly added 15 cc. of nitric acid (sp. gr. 1.2).
The mixture is permitted to stand for 24 hours and then filtered. The
filtrate should have a yellow color and immediately precipitate any
twentieth normal phosphate solution.

Solution II is a 1 or 2 per cent solution of phenylhydrazine hydro-
chloride. Equal parts of solutions I and II are mixed just before use.
The phosphates take on a blue color.

Iodine. (Justus, Virchows Arch., 1902, clxx, 501.) Fresh unfixed
tissue is cut in frozen section and placed for ten minutes in a green
colored chlorine water which is kept in a closed glass bottle. It is then
placed for three hours in the dark in the following mixture: 1 cc. 1 per

cent silver nitrate, distilled water 500 cc.   The tissue is then hurriedly washed in distilled water and placed in a warm saturated solution of sodium chloride for three hours, then again washed in distilled water and placed for from one to three minutes in a 5 per cent solution of mercuric chloride, and finally mounted in glycerin.   The iodine appears as reddish brown granules.

Sodium.   The method here described was devised by us and is based upon the Kramer-Tisdall method for determining sodium in the blood. Fresh pieces of tissue are frozen, cut, and placed in the reagent for ten minutes.   The sections are then placed in 80 per cent alcohol and mounted in glycerin.   The sodium appears as a pyroantimonate in crystalline form.

The reagent is prepared as follows: To 500 cc. of boiling distilled water add 10 gram of potassium pyroantimonate, and continue heating for five minutes, then plunge the flask into cold water until cool.   When cool add 15 cc. of a 10 per cent solution of potassium hydroxide (purified by alcohol) and filter into paraffin lined bottles.   Of this stock solution, take 10 cc. and dilute up to 30 cc. with 10 per cent formalin, neutralized with an alkali which is sodium free; then add 9 cc. of 95 per cent alcohol.

Calcium.   (Macallum, ibid.)   Fresh tissue is frozen, cut, and placed for twenty minutes in sulphuric acid (sp. gr. 1.84) 2 cc., absolute alcohol, 100 cc.   It is then thoroughly washed in absolute alcohol and placed in a tenth normal solution of lead acetate for ten minutes, after which it is washed for thirty minutes in distilled water, placed in $N/10$ HNO_3 for a few minutes, again washed in distilled water, and mounted in glycerin ammonium sulphide (see potassium).   The calcium deposits are black.

Iron.   The inorganic forms of iron were demonstrated with the technic giving the Prussian blue reaction.   For the masked iron deposits the method advocated by Macallum was employed.   The tissue is fixed in alcohol for a few hours and cut, flooded with ammonium sulphide and covered with a cover glass (the edges of which are sealed with balsam).   The slide is then placed in the incubator at 37°C. for from three days to two weeks.   After two days the iron granules begin to turn green, the color gradually becoming darker.

Copper.   The method used for the demonstration of masked iron was employed; copper, it is stated, appears as a brown precipitate and iron as a black one.

Glycogen.   The technic described by Best was employed.

Intracellular ferments. (Unna.) Fresh unfixed tissue is cut in frozen section and stained for from five to ten minutes in an 0.5 per cent aqueous solution of neutral violet extra (Grübler). It is then washed in tap water, differentiated in 95 per cent alcohol, and cleared in oil of bergamot. Areas in which oxidation is occurring are said to be stained red, while those in which reduction is taking place are blue.

Olein and oleic acid. The tissue is fixed in formalin and cut on a freezing microtome. Sections after thorough washing are placed for twenty-four hours in a 1 per cent osmic acid solution, then washed in water for several hours, and mounted in glycerin. Both substances become brownish black or black.

Neutral fats. After fixation in 10 per cent formalin, frozen sections are stained for ten minutes in a concentrated aqueous solution of Nile blue sulphate, then differentiated in a 1 per cent solution of acetic acid, thoroughly washed in water, and mounted in glycerin. Neutral fats stain light red while fatty acids appear as glistening blue drops.

Cholesterol. Fresh tissue is cut, frozen, and flooded with formalin 30 per cent, 2 cc., sulphuric acid, 5 cc. After one or two minutes the cholesterol becomes brownish red.

Lipoids. The usual Sudan III staining technic for lipoids was employed. (Ciaccio: Centralbl. f. allg. Path. u. path. Anat., 1909, xx, 385–771.) Small pieces of tissue were fixed for twenty-four hours in formalin 10 per cent, washed thoroughly in water and then placed for from five to eight days in a three per cent potassium bichromate solution.

After this, the fragment of tissue is washed for twenty-four hours in running water and treated as follows:

	hours
Absolute alcohol	2
Absolute alcohol and xylol equal parts	1
Xylol	1
Saturated solution of paraffin and xylol	2
Paraffin	1

Embed and cut.

Stain, after elimination of the paraffin, in a saturated solution of Sudan III in 70 per cent alcohol where the slides are left for about thirty minutes. Then they are rapidly washed in 60 per cent alcohol followed by distilled water and mounted in Apathy's gum acacia.

Broadly speaking, the various substances sought for may be divided into those always present in or about a cell, and those

which may or may not be present.   As to the protein constitu-
ents of the cell the present investigation offers no data, though it
is currently accepted that the cell consists of a protein gel in
which other substances may or may not be present.

Lipoids.   Lipoids were found to be very irregularly distributed,
and when present were either intra- or extracellular or nuclear.
Necrotic areas and zones of active proliferation showed much
more lipoid deposit than did healthy areas which were not actively
proliferating.   Since tumor tissue is both more actively proliferat-
ing and degenerating than normal tissue, it was not surprising to
find a greater deposit of lipoid substances in neoplastic tissue.
The various lipoid fractions showed interesting variations.

Cholesterol as such was not demonstrable in any of the tissues
examined; and though the method employed was one of the
crudest of the various color reactions given by this substance, it
sufficed to show cholesterol deposits in a gouty tophus used as
control.   Although cholesterol has been obtained from all types
of tissue, the fact that it is not soluble in any of the body juices or
fluids renders it very probable that in the cell it does not exist as
free cholesterol.

Olein and oleic acid were found in the cells of the areolar con-
nective tissue, but were not seen in other healthy cells of either
normal or neoplastic tissues.   These two lipoid fractions were
encountered only in necrotic areas and in degenerating cells
adjacent to necrotic areas and when present were either intra-
or extracellular, but not in the nucleus.   Since receding tumors
contain large amounts of necrotic material, both olein and oleic
acid were more frequently encountered in them than in other
tissues, though there was no apparent increase in the relative
amounts present in benign or malignant necrotic tissue, when
the surface areas of the necrotic portions were of similar extent.

In the cytoplasm of both normal and neoplastic cells showing
one or another phase of mitosis, there were numerous large
granules giving the staining reaction for fatty acids (fig. 1).
Fatty acids, oleic acid excepted, were not encountered in
degenerating cells, though in these cells, whether they were
malignant or normal, neutral fats were not infrequent.   The

fatty acids and neutral fats were found intracellular and extra-nuclear. Rapidly dividing tissues had the larger amounts of fatty acids, while neutral fats were more abundant in degenerated areas. In these respects no differences between normal and neoplastic tissue were evident.
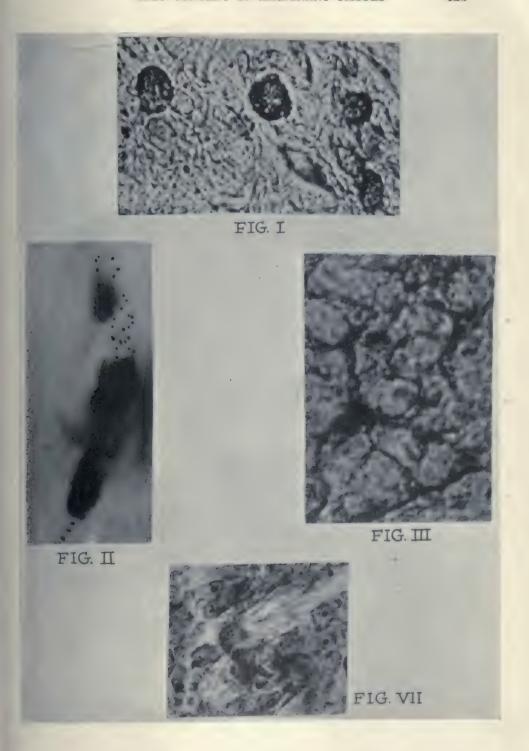
Ferments. Dividing cells in general, irrespective of type, showed within the cytoplasm, but not in the nucleus, granules which took the stain supposedly indicative of the presence of oxidizing reactions (fig. 2). These granules also gave the reaction said to be characteristic for fatty acids. In neoplastic tissues the granules were larger than in the normal. Healthy cells not undergoing division showed no granules. In cells undergoing degenerative changes the reaction supposedly indicative of reduction processes was present.

Iodine. With the technic employed there were found in the cells of both normal and neoplastic tissue tiny black or dark yellow-red granules in the position of the nucleolus. Similar granules were found in the intercellular spaces of the rat thyroid gland. Aside from the fact that in neoplastic cells these granules were larger, there were no differences in the various types of tissue examined.

These granules were present in such numbers that one would expect a qualitative reaction for iodine when a larger bulk of the tissue is ashed and tested. Upon trial, however, we were not able to demonstrate the presence of iodine qualitatively when large amounts of tissue were examined, hence this reaction is probably valueless.

Iron and copper. Inorganic iron was demonstrable only in such areas as had been the site of hemorrhage. Organic iron could occasionally be demonstrated in the cytoplasm of some of the cells in both normal and neoplastic tissues. It was not a

FIG. 1. GRANULES OF FATTY ACIDS IN DIVIDING CELLS

FIG. 2. OXIDIZING GRANULES IN CELLS UNDERGOING MITOSIS

FIG. 3. CHLORIDE DEPOSIT ABOUT THE CELL WALL

FIG. 7. SODIUM DEPOSITS WITHIN CELLS

FIG. I

FIG. II

FIG. III

FIG. VII
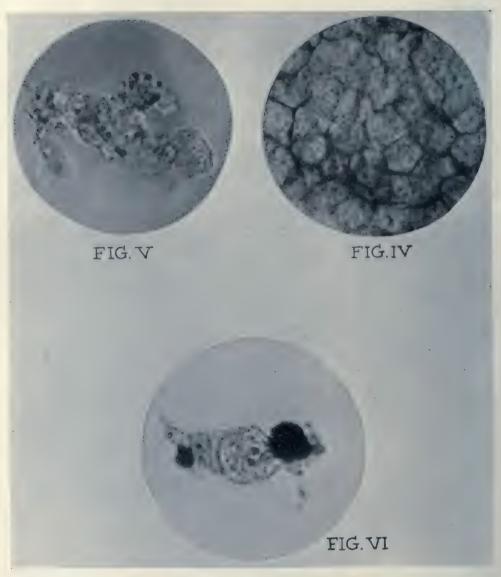
FIG. 4. SULPHATE DEPOSIT ABOUT CELL WALL
FIG. 5. CALCIUM DEPOSITS WITHIN CELLS
FIG. 6. POTASSIUM DEPOSITS WITHIN CELLS

constant element and there was no decided difference between malignant and normal cells. We were unable to demonstrate copper in our sections, although recently (1) copper has been found in malignant growths.

Glycogen. The amount of glycogen found seemed to increase with the proliferative activity of the tissue, but all cells did not contain it, even in the tissues in which glycogen was most abundant. When present the granules were intracellular. Sarcomata contained more than carcinomata; and receding tumors less than either growing normal tissue or growing malignant tissue.

Phosphates, chlorides, and sulphates. Inorganic phosphates were occasionally encountered as isolated extracellular masses in both normal and neoplastic tissue, though in some receding tumors similar aggregations were present within the cell. There was no apparent dominance of inorganic phosphates in any of the tissues examined. Organic phosphates were present as diffuse deposits in both cell cytoplasm and nucleus without characteristic variations in quantity.

In both benign and malignant tissues the chlorides were distributed either in the wall of the cell or between the cells (fig. 3); only rarely, and then only in degenerating cells, were they demonstrable within the cytoplasm.

The sulphates were distributed in a manner identical with that of the chlorides and, as with the former, only within the cell in degenerating elements (fig. 4).

That both potassium and calcium show quantitative differences in rapidly growing and in slowly growing tumors is well established through the work of Beebe and Buxton (2) for human tumors, and Frisbie and Clowes (3) for animal neoplasms. While the results recorded here confirm the work of these investigators the present data are novel in some particulars.

Calcium is demonstrated in the nucleus as a fine needle-like precipitate, while that in the cytoplasm is finely granular. Large coarse granules are found between the cells. The ratio of calcium within the cell to that without the cell is approximately as 1 to 20 (fig. 5).

Potassium is demonstrable in the cytoplasm of the cell as a granular deposit, but is absent in the nucleus (fig. 6). The ratio of potassium within the cell to that outside the cell approximates 1 to 5.

Sodium (fig. 7) may be demonstrated as a crystalline deposit in the spaces between the cells and in the nucleus. When the cell is degenerating it is found in the cytoplasm.

While the technical methods employed precipitated the various elements in definite places in the cell, it does not seem reasonable to suppose that during the life of the cell these elements exist in the manner present in the microscopic preparations. It is more probable that they exist in solution, which in turn presupposes a certain degree of ionization or of protein combination. A careful comparison of many fields and many slides suggested variations in the potassium, sodium, and calcium content of tumors in various phases of their growth, though it is needless to say that the crude methods employed warrant no definite conclusions.

The differences observed in our microchemical preparations led to further investigations, in which the potassium, sodium, and calcium content were determined after the method described by Kramer and Tisdall (4).

The blood of the following groups of animals was examined for its potassium, sodium, and calcium content: rats bearing progressively growing Jensen sarcoma, receding Jensen sarcoma, Jensen sarcoma which had completely receded, spontaneous sarcomata of the liver (Bullock and Curtis stock); rats injected with living rat spleen; with dead rat spleen; and rats naturally resistant to the growth of the Jensen sarcoma.

Beside making a blood analysis, the salt content of the following tissues obtained from the same groups of animals was estimated: progressively growing Jensen sarcoma, receding Jensen sarcoma, spontaneous liver sarcoma (Bullock and Curtis stock), normal rat liver, liver from bearers of spontaneous rat liver sarcoma (using that portion of the organ not involved in the neoplasm), normal testes, testes from bearers of progressively growing Jensen sarcomata, testes from bearers of receding Jensen sarcomata.

The numerical data for each group are given in table 1.

It would have been ideal to make these determinations upon the various animals during the course and development of the tumors, so that the results would actually portray what was occurring during the various phases of cell growth in a given animal. Unfortunately, however, the amount of blood required rendered it impossible to save the animal. The averaged results

TABLE 1

GROWING JENSEN SARCOMA			NORMAL RAT LIVER		
K	Ca	Na	K	Ca	Na
292	10	302	162	5	56
319	10	156	225	4	123
205	9	319	224	12	199
257	7	311	315	8	286
255	3	248	284	6	200
338	6	263	168	2	166

TESTES OF RAT WITH GROWING JENSEN SARCOMA			TESTES OF RAT BEARING RECEDING JENSEN SARCOMA		
77	4	94	86	4	165
80	7	254	294	1	148
59	4	102	223	4	75
103	7	205	204	9	65
228	1	209	356	2	458
234	3	166	340	3	313
123	6	190			

TESTES OF NORMAL RAT			LIVER OF RAT WITH SPONTANEOUS SARCOMA		
293	2	321	49	11	480
280	6	271	69	15	523
104	1	272	255	14	235
31	3	139	233	18	375
178	1	95	264	27	302
110	3	66	235	1	142

RECEDING JENSEN RAT SARCOMA			SPONTANEOUS SARCOMA RAT LIVER		
32	2	172	225	9	248
41	4	255	215	7	260
92	11	481	258	8	269
203	1	121	342	11	238
171	3	123	171	3	171
164	2	96	160	6	192

TABLE 1—*Continued*

BLOOD OF RAT WITH SPONTANEOUS LIVER SARCOMA			NORMAL RAT BLOOD		
K	Ca	Na	K	Ca	Na
89	4	291	178	5	241
84	4	238	176	5	203
85	4	325	175	4	225
96	6	302	193	6	247
135	5	236	141	6	177
59	5	246	134	5	189
			155	7	204
			186	5	218
			165	6	228
			147	6	266
			186	3	158
			180	3	197

BLOOD OF RAT BEARING GROWING JENSEN SARCOMA			BLOOD OF RAT INJECTED WITH EMULSION OF LIVING RAT SPLEEN CELLS		
277	13	97	161	5	187
184	9	166	155	9	171
118	3	276	136	7	168
60	2	197	161	8	180
72	5	178	137	6	165
134	4	208	162	5	200

BLOOD OF RAT BEARING RECEDING JENSEN SARCOMA			BLOOD OF RAT INJECTED WITH EMULSION OF DEAD RAT SPLEEN CELLS		
139	5	226	136	3	248
138	4	218	146	3	221
114	5	383	140	3	305
95	6	406	107	4	210
87	2	398	204	3	314
82	2	457	137	5	191
			214	4	294
			150	5	247

BLOOD OF RAT IN WHICH JENSEN SARCOMA HAS COMPLETELY RECEDED					
142	7	257			
161	4	229			
112	5	226			
130	5	213			
126	6	222			
121	4	227			

All values are given as milligrams per 100 grams of wet tissue or per 100 grams of blood.   Fractions of a milligram have been discarded.

in a given group, however, serve to indicate conditions in an average animal, so that we may accept as the normal values the averaged value obtained for the normal group; while the growing, receding, and completely receded Jensen sarcomata groups, as averaged, represent the conditions during the various phases of that tumor strain's growth and recession. The spontaneous liver sarcomata group shows the condition during the growth of a spontaneous tumor. In accordance with this idea, the averaged group behavior is shown graphically in charts 1, 2, 3, and 4, the numerical data being given in table 1.
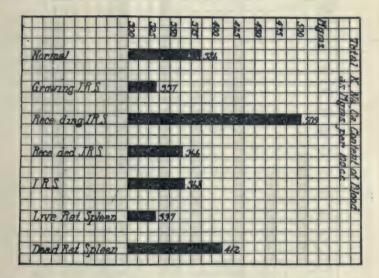
CHART 1. TOTAL K, Na, Ca CONTENT OF BLOOD AS MILLIGRAMS PER 100 CC.

As is shown in chart 1, when malignant cells are parenterally introduced (as occurs with intentional inoculation) and grow, there occurs a demineralization of the blood.[1] Of more signifi-

[1] The growing Jensen sarcomata which were examined were taken when four weeks old and only after they had given conclusive evidence of progressive growth. The receding Jensen sarcomata were taken, 3 at the start of the recession, and 3 after recession was well developed, the tumor having diminished to one-half of its largest size or less. The animals injected with either living or dead rat spleen pulp were injected subcutaneously with a dose of 0.5 cc. and examined ten days after injection. The cells in the dead spleen emulsion were killed by boiling.

cance is the fact that an analogous demineralization occurs when
the malignant cells develop in the host, and also when benign
living cells are introduced parenterally. When these cells
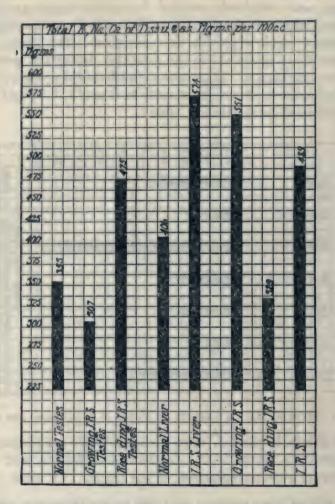
CHART 2. TOTAL K, Na, Ca OF TISSUE AS MILLIGRAMS PER 100 CC.

die (as occurs with a spontaneously receding tumor), or are
introduced dead (as when an emulsion of dead rat spleen was
injected) there is an accumulation of the salts in the blood stream.

When the dead cells are completely absorbed, as in the group in
which the Jensen tumor has completely receded, the salt content
of the blood shows a return to the normal.
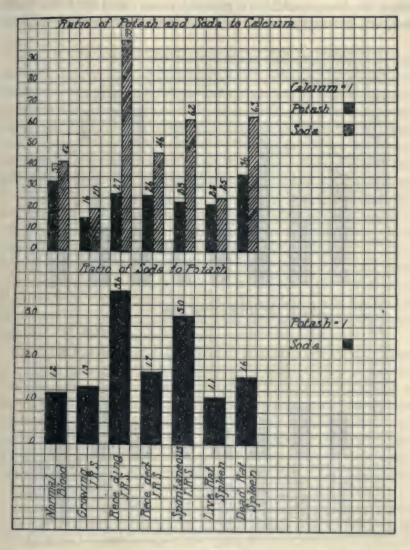
CHART 3. SHOWING RATIO OF POTASSIUM AND SODIUM TO CALCIUM AND OF SODIUM
TO POTASSIUM IN THE BLOOD

While these changes are occurring in the blood, analogous changes take place in the tissues (chart 2). When testicular tissue is analyzed, it is found that the salt content diminishes when the bearer exhibits a growing tumor, and that the organ recovers its earthy constituents as the tumor recedes, while in
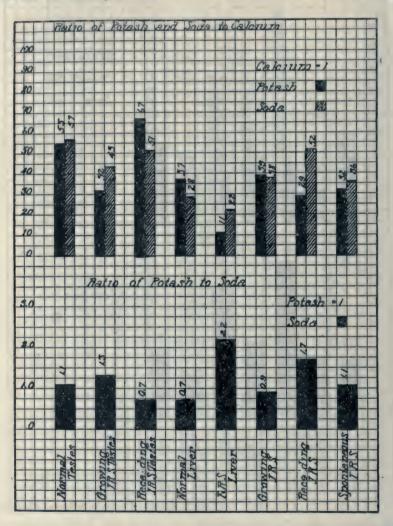
CHART 4. SHOWING RATIO OF POTASSIUM AND SODIUM TO CALCIUM AND OF SODIUM TO POTASSIUM IN THE TISSUES

the tumor itself there is a very marked demineralization as recession goes on.   When the tumor is spontaneous in origin, the organ in which the neoplasm arises (in this instance, liver) becomes more mineralized than the normal liver, while the neoplasm contains less mineral than do the surrounding non-malignant portions of the organ.

With the changes in the total sodium, potassium, and calcium content, there occur variations in the ratios existing between these salts.   If the blood of rats bearing growing Jensen sarcomata (see chart 3) is compared with the normal, the calcium content being taken as 1, there is found a diminution to an almost equal degree in both the potassium and sodium ratio.   When the tumor commences to recede there is an increase in the sodium ratio, and when the tumor has completely receded the sodium returns to the normal ratio.   When non-malignant cells are introduced the changes are of the same type, but not so marked.   Similarly, when benign cells are injected dead, we observed the same type of disturbance as when malignant cells die spontaneously.   The blood of rats bearing a spontaneous tumor shows the same type of change observed in those with a receding tumor or in the group injected with dead benign cells, which might be explained on the basis that in these spontaneous liver sarcomata there are large amounts of necrotic material.

In the fixed tissues of the body the changes in the various ratios are of the same order as in the blood.   Thus (see chart 4) in the testicle, with the progressive growth of a tumor there occurs a decrease in the potassium and sodium ratio exactly as in the blood, and when the tumor spontaneously recedes there is an increase of the potassium and, to a lesser extent, of the sodium. In the tumor itself, when it is progressively growing, the potassium and sodium ratio are almost equal, but when the tumor recedes the potassium ratio falls and the sodium ratio rises.

In the tissues of the spontaneous tumor bearer, the liver, where it is not involved in the tumor process, shows a decrease of both the potassium and sodium ratio, though the total salt content is much increased when compared with a normal liver.   The tumor itself shows a ratio of potassium and sodium very close to that of the growing transplanted Jensen sarcoma.

It would appear, judging from the results of the experiments recorded in this paper, that the physicochemical composition of the cell is somewhat on the following order. The cell may be considered as a gel containing within it a more compact gel, the nucleus. In the denser gel calcium and sodium are present, while in the cytoplasmic gel potassium and calcium are present, the former dominating. These metals are present as phosphates, the chlorides and sulphates being present in the cell wall. Calcium, potassium, and sodium are present in the fluids about the cell. Lipoid or lipoid fractions appear when the cell is either dividing or degenerating; if present at other times they are in such combination that their staining reactions are masked. Oxidizing and reduction processes which occur within the cell are associated, physically at least, with fatty acids. Glycogen appears only occasionally and is possibly also associated with the oxidizing processes which accompany mitosis.

Since the metals were the only elements present at all times in all tissues, they are the only substances on which comparisons are possible. It might be suggested, on the basis of the Donnan equilibrium theory, that the presence of salts in various portions of the cell gel and surrounding fluids, plus the demonstrated changes in the blood of animals showing various biological phases of tumor development ((growth and recession), would account for a flow of ions from one part of the cell gel to another, or from without the cell into the cell or vice versa, and on such a basis an attempt might be made to explain the unrestrained growth properties of the malignant cell.

Unfortunately the facts shatter the theory, for we find both benign and malignant cells inducing the same type of salt change. It is therefore reasonable to suppose that the changes observed are secondary to cell growth and degeneration and not the cause of it.

Before describing the further investigations, it may be profitable to review briefly some of the facts already known concerning mineral metabolism in its relationship to neoplasia.

Clowes and Frisbie, and Buxton and Beebe have shown that slowly growing tumors contain less potash and more calcium than rapidly growing ones. Later Clowes claimed that the injection of potassium salts increased the susceptibility of the animal to metastasis. Goldzieher (5) reported that calcium decreased the rate of growth of young animals, the inhibitory action of calcium being subsequently confirmed in another fashion by Cramer (6). Cattley (7), who studied benign and malignant cells microchemically, found the microdistribution of calcium the same in both types of cell. Jensen (8) found that the calcium content of the blood varied with the age, decreasing progressively decade by decade, being 12.46 at the twentieth year, and 10.95 at the fiftieth; in other words, showing a progressive decrease in the so-called cancer age period.

A query which arises is the possibility that the dominance of potassium in a tissue might predispose to neoplastic development if a chronic irritant were simultaneously applied. On this basis attempts were made to increase the potassium content in the blood of rabbits by intravenous injections of monobasic potassium phosphate, a chronic irritant (Scharlach R) being injected in

TABLE 2

	K	Ca
Site injected with potassium............................	93	32
Site injected with calcium............................	75	87
Control site..	74	31

All values as milligrams per 100 grams of wet tissue.

the ear. These attempts resulted negatively and it was considered possible that the salt was not taken up by the tissues. Therefore a series of 6 rats were injected in the right flank with a 5 per cent solution of monobasic potassium phosphate, and in the opposite flank with a 5 per cent solution of calcium lactate. After five such daily injections the animals were killed. The respective sites of injection were removed, together with the pectoral muscles as controls, and the amounts of potassium and

calcium determined in each sample.   The averaged data given in
table 2 show that a fraction of the injected salt remains fixed in the
tissues into which it is injected.   The tissues of the breast in
rats were then injected with sodium in one series, potassium in
another, and calcium in a third.   The salt content was kept high
by repeated injections, and in this area Scharlach R was injected
as the irritant.   The tissues of these animals showed no changes
which were not also observed in the controls.
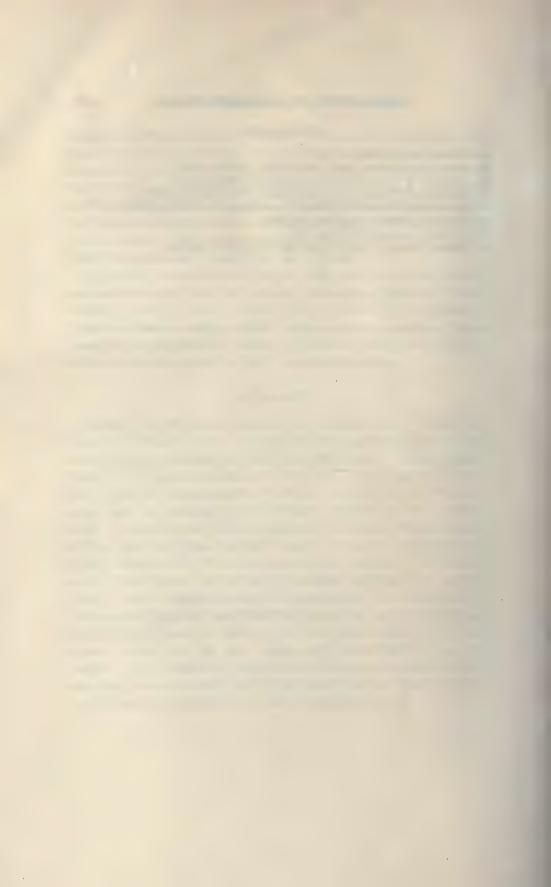
Injections of the various soluble salts of potassium, sodium,
and calcium, also failed to cause any recession of growth in mice
bearing Crocker Fund sarcoma 180.   Other mice repeatedly
injected before inoculation with these same salts and then inocu-
lated at the injected area, failed to show the development of any
resistance to the growth of this same tumor strain.

CONCLUSIONS

A study of the microchemistry of malignant and benign rat
tissue cells is presented.   It is shown that demonstrable changes
occur in the salt content of the tissues and blood.   These changes
consist in a demineralization of the blood and tissue cells upon
the parenteral introduction of living cells irrespective of their
type, with an increase of the mineral content of the growing
cells.   A further study shows that upon the death and absorption
of the cells they give up their mineral content which again is
rapidly taken up by the body tissues and the blood.   There also
occur disturbances in the ratios existing between the various
salts.   These changes in the salt metabolism do not apparently
influence the origin of neoplasms, nor have they any demonstrable
effect upon immunity against the transplantation of rat and
mouse tumors, nor do they cause such transplanted tumors to
recede.   It is therefore probable that the changes observed are
secondary to cell growth and death, and bear no relationship to
the biological character of the cells which grow or die.

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